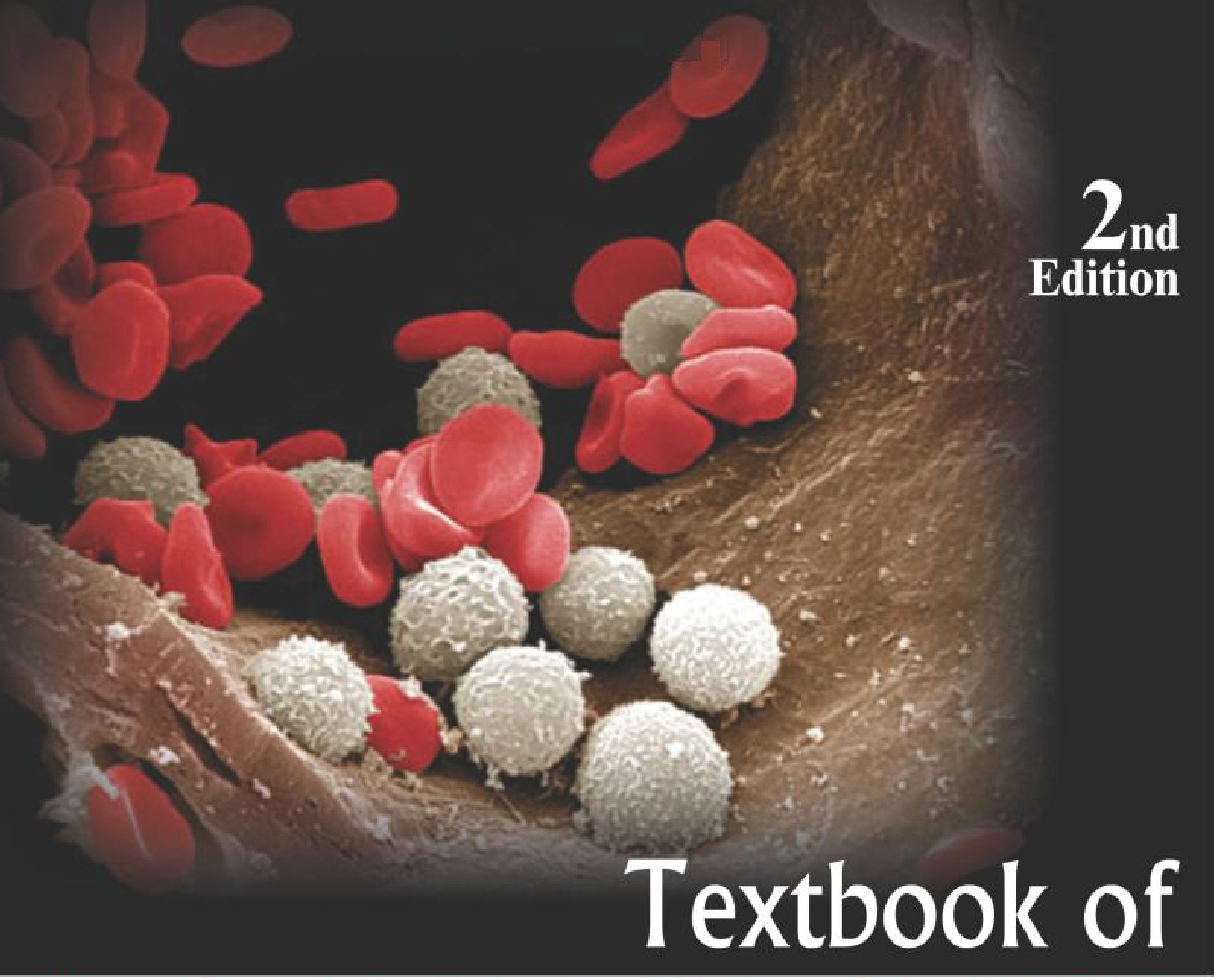

2nd
Edition

# Textbook of
# IMMUNOLOGY

**Sunil Kumar Mohanty**
**K Sai Leela**

*Foreword*
**KC Nathsarma**

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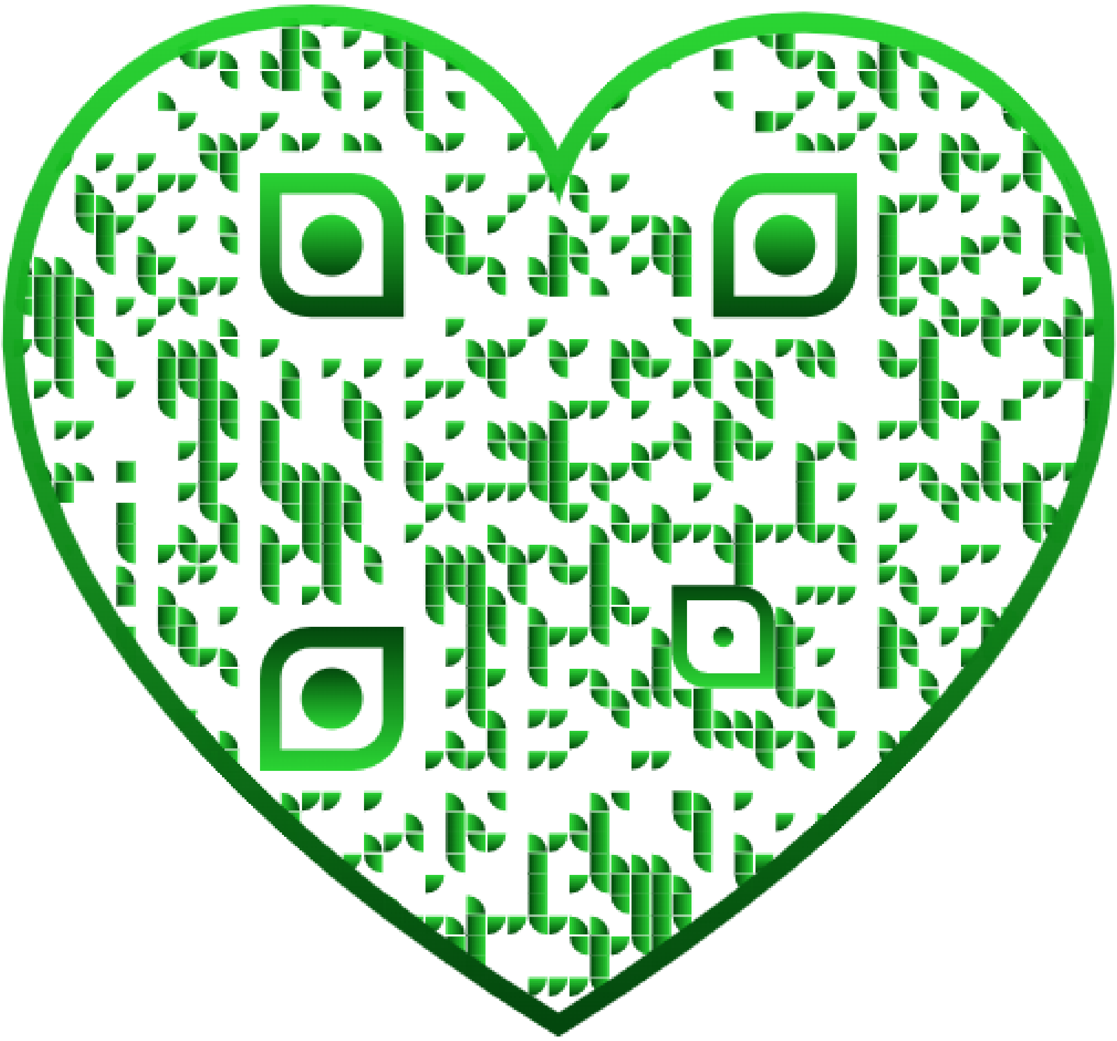

# Textbook of
# Immunology

# Textbook of Immunology

Second Edition

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## Dedicated to

*The loving memory of my colleague*
*Late Dr Subrat Mishra*
*whose inspiration and encouragement*
*prompted me to write a book on immunology*

—***Sunil Kumar Mohanty***

*The teachers and the taught*

—***K Sai Leela***

# Foreword

It is my immense pleasure to write the foreword to the *Textbook of Immunology* authored by Dr Sunil Kumar Mohanty and Dr K Sai Leela.

The immunology as a subject has grown immensely in the last 50 years. The amount of detail knowledge that has been accumulated has already reached paroxysmal proportion. If there has been a remarkable progress, then it is in the domain vaccinology, which led to the control of a number of infectious diseases. But, there remains a crying need for vaccine against others. It is hoped that the immunologists of today, using the tools of molecular and cellular biology, genomics, and proteonomics will make inroads in preventing these diseases. Presently, the immunodiagnostic measures are of great utility in diagnosis and monitoring the treatment of various diseases. The *Textbook of Immunology* serves a great purpose not only in understanding the fundamentals of immunology but also to delve into the depth to explore the fascinating aspects of the immune system, that will make them doctors, researchers and teachers. Very few books are complete in all aspects.

The authors have made every effort to present the text in a lucid and readable style with illustrative graphics and colored diagrams without sacrificing the important details.

I congratulate the authors for the compendium, which is bound to be of great interest and utility for students, researchers, teachers and doctors of medical and other allied sciences.

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# Preface to the Second Edition

The goals for the second edition of *Textbook of Immunology* have remained the same as those of the first edition published in 2007 to provide a brief, accurate and updated presentation of those aspects of immunology that are of particular significance in the fields of clinical infections, diagnostic immunology, vaccinology, transplantation immunology and in other allied fields of biological sciences.

In the last few years, we have received certain comments from our readers, expressing satisfaction on some parameters and also pointing out certain inadequacies. We acknowledge their inputs with humility and utmost respect and have acted on the shortcomings meticulously in the second edition of *Textbook of Immunology*.

Initially, the book was designed, mainly, for the use of undergraduate students. Therefore, we stressed more on basics than going deeply into the intricacy.

In honoring the recommendation of a number of learned teachers, in the fields of immunology, we have not only included certain topics such as HIV infection (AIDS), immunology of fungal infections, etc. but also updated the knowledge on various subjects such as antigen receptors, cytokines, their receptors and future prospects, 'B' cell and 'T' cell repertoires, autoimmune disease, etc. Almost in every chapter, there has been addition, deletion and correction of inadvertent errors without compromising the lucidity, clarity and consistency. We have also included some more figures and tables wherever required.

Perfection is too vast for comprehension. But striving for perfection is the triumph of human intelligence. We have worked hard to minimize the shortcomings. However, we fervently desire to get the regular inputs from our readers to smoothen the angularities.

We have consulted friends, especially, Dr SS Mishra, Professor of Pharmacology; Dr Shruti Mohanty, Professor of Biochemistry, and colleagues with relevant expertise to advise us and to those we owe our sincere gratitude.

We hope that the second (revised) edition of *Textbook of Immunology* will invite today's students not only to acquire familiarity but also to delve into the depth to explore the fascinating aspects of the immune system, that will make them doctors, researchers and teachers.

**Sunil Kumar Mohanty**
**K Sai Leela**

# Preface to the First Edition

Basically, we reside in a hostile world amidst bewildering array of infectious agents of diverse size, shape and subversive character. If we had not developed an immune system against these, they would have made us a sanctuary for propagation of their selfish genes. But the provision of providence is such that though these tiny organisms live in, on and around us, under normal circumstances, they cannot establish infection. They are being eliminated by the components of immune system.

The immunology as a subject gained its importance in the last one or two decades. Virtually, there has been an explosion of knowledge in the field of immunology. The explosion not only provides a greater understanding in the mechanism of elimination of infectious and noninfectious foreign invaders but also imparts enough knowledge about various immunopathological disorders. Presently, the immunodiagnostic measures serve a great purpose in diagnosis and monitoring the treatment of various diseases. The vaccines play important roles in preventing communicable diseases and reducing the morbidity and mortality.

As a teacher of immunology, I had the privilege to delve into the minds of the undergraduate students as regard to their understanding of the subject of immunology and their application in various fields such as research, diagnosis and therapy. At undergraduate level, students learn immunology as a part of microbiology. The various textbooks which are in use, spare a section for immunology. The chapter on immunology in textbooks of microbiology lacks in certain essential aspects. In some books, though the information is sufficient and the language is lucid, the diagrammatic representations are not sufficient or poor or not up-to-date. On the other hand, in some books, though the diagrams are better, the contents are so precise that they do not convey proper meaning. Very few books are complete in all aspects. We have made every effort to write in a lucid readable style with illustrative graphics and colored diagrams, without sacrificing the important details.

This book contains chapters on the History of Immunology, Basic Immunology, Diagnostic Immunology, Immunopathological Disorders, Immunohematology, Transplantation Immunity Against Bacteria, Parasites, Viruses and Fungi. We felt the need of inclusion of host-parasite relationship in our books as the students should have a basic knowledge about the determinants of pathogenicity before they study the immune response produced against them. This book would, thus, naturally span the curricula of medical, dental and other allied courses both basic and applied.

It would have been impossible to be authoritative about every aspects of immunology. For this reason, we have consulted friends and colleagues with the relevant expertise to advise us and to those we owe our gratitude. Despite all our efforts, some omissions, and inadvertent errors might exist. In that case, those may kindly be brought to our notice for supplementation or correction in subsequent edition.

We hope that the first edition of *Textbook of Immunology* will invite today's students to acquire a familiarity with immune system that will make them doctors, researchers and teachers.

**Sunil Kumar Mohanty**
**K Sai Leela**
**P Veerendra Kumar Reddy**

# Acknowledgments

The authors are deeply indebted to many dedicated and talented professionals who generously spared their time and expertise to make the publication of second edition of the book possible. Of them, Dr SS Mishra, Professor of Pharmacology, Institute of Medical Studies (IMS), Bhubaneswar, Odisha, India, and Dr Shruti Mohanty, Professor of Biochemistry, Kamineni Institute of Medical Sciences (KIMS), Narketpally, Nalgonda, Andhra Pradesh, India, deserve special mention for their expertise and invaluable artistic talents. Dr Dipti Pattnaik, Professor of Microbiology, Dr Bandana Mallick, Professor of Microbiology and Mr Priyaranjan Lenka, Tutor of Microbiology of Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, have rendered their keen outlook and expertise in making of this book, but without their contribution, it would not have been possible. We gratefully acknowledge all colleagues and postgraduate students of KIMS, Bhubaneswar, and KIMS, Narketpally, for their valuable suggestions and assistance in preparation of the manuscript. We thank our computer assistants, Mrs Bhagyalaxmi and Mr Arun, for their sincere efforts in typing the text matter.

We also take the opportunity to thank Shri Jitendar P Vij (Group Chairman), Mr Ankit Vij (Managing Director) and Mr Tarun Duneja (Director-Publishing) of M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, for their relentless, unstinted and unwavering support.

Finally, we appreciate our families and friends for their whole-hearted cooperation.

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# A Short History of Immunology

# 1

The discipline of immunology grew out of the observation that individuals who had recovered from certain infectious diseases were, there after, protected from the disease. Perhaps the earliest written reference to the phenomenon of immunity can be traced back to Thucydides, the greatest historian of the Peloponnesian war. In describing, a plague in Athens, he wrote in 430 BC that only those who had recovered from the plague could nurse the sick, because they would not contract the disease a second time.

The first recorded crude attempts to induce immunity deliberately were performed by the Chinese and Turks in the 15th century. Various reports suggest that the dried crusts derived from smallpox pustules were either inhaled into the nostrils or inserted into small cuts in the skin (a technique called variolation). In 1718, Lady Mary Wortely Montagu, the wife of British Ambassador to Constantinople, observed the positive effects of variolation on the native population and had the technique applied to her own children. The technique was significantly improved by the English physician, Edward Jenner in 1798. Intrigued, of the fact, those milkmaids who had contracted cowpox (a mild disease) were subsequently immune to smallpox. Jenner reasoned that introducing fluid from a cowpox pustule into people might protect them from smallpox. To test this idea, he inoculated an 8-year-old boy with fluid from a cowpox pustule and later intentionally infected the child with smallpox. As predicted, the child did not develop smallpox. Jenner's technique of inoculating with cowpox to protect against smallpox spread quickly through Europe.

Even though, the immunology was born in the 18th century with the practice of variolation and of Jenner's vaccination, it became a true science only in the 19th century. Beginning in 1880, the world witnessed a veritable torrent of progress, stimulated both by Pasteur and his students and by Robert Koch and his group. The magnificent contribution was done by Pasteur, Roux, Metchnikoff and Bordet on the one hand and those of Koch, Pfeiffer and Ehrlich on the other. Louis Pasteur showed with his work on fowl cholera, anthrax and rabies that these organisms once attenuated, could be used specifically to protect the individual against the disease that they caused. From this, extraordinary series of investigations were born, the modern science of immunology. In 1885, Pasteur administered the first vaccine to a human, a young boy, who had been bitten repeatedly by a rabid dog (Fig. 1.1).

Emil von Behring and Kitasato in 1890, inoculated toxins of diphtheria and tetanus to animals, to produce neutralizing antitoxin serum. They introduced passive immunization in modern medicine for which von Behring was awarded Nobel Prize in 1901.

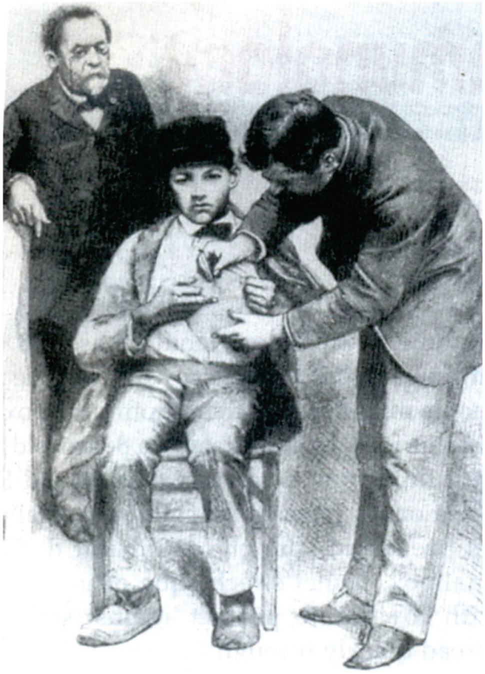


**Fig. 1.1:** Wood engraving of Louis Pasteur watching Joseph Meister receiving the rabies vaccine by a third person (From Harper's weekly 29:836; Courtesy of National Library of Medicine)

The phagocytic theory was first to be conceptualized in 1880 by a Russian zoologist named Elie Metchnikoff. He hypothesized that the basis of inflammation was the cellular reaction. Vascular and nervous reactions were secondary. He further suggested that the elimination or destruction of invaders was the sole function of phagocytic cells. Subsequently, many workers proposed the presence of soluble substances (humoral factors), which were bactericidal. Finally in 1903, Almroth Wright and Stewart Douglas discovered a humoral component known as opsonin, which makes the target bacteria palatable for phagocytosis. In 1908, Metchnikoff and Ehrlich shared the Nobel Prize for the respective studies of cellular and humoral factors in host defense. Ehrlich proposed side-chain theory of antibody formation and antibody function.

In 1905, von Pirquet and Schick published a brilliant series of studies on children treated with horse diphtheria antiserum, which led to untoward reaction. They then introduced the concept that all the events in immunological reactivity might not be beneficial to the host and they named the disease caused by the immune response to horse protein 'serum sickness' a term still in use. In the same decade Arthus, Proteir and Richet described the cutaneous vasculitis and anaphylaxis in animals. In 1913, Charles Richet received the Nobel Prize in recognition to his work on anaphylaxis.

Pfeiffer discovered the phenomenon of *in vivo* cytolysis of *Vibrio cholerae* much earlier (1894–1895), following intraperitoneal inoculation of *V. cholerae* in an immunized guinea pig. This early experiment led to the understanding of complement-mediated cytolysis by Pfeiffer and later Buchner and Bordet. Jules Bordet was honored with the Nobel Prize in 1919 for his work on complement.

Being successful in passive immunization against diphtheria and tetanus von Behring applied this conception in tuberculosis and failed miserably. Robert Koch also had to accept the failure in providing immunity against tuberculosis. Ultimately, Albert Calmette and Camille Guérin, the French scientists found out an effective vaccine Bacille Calmette-Guérin (BCG) for tuberculosis (1921). The vaccine had been prepared from the attenuated strain of *Mycobacterium bovis.*

Twentieth century witnessed phenomenal advances in understanding the immunological concepts. As the subjects grew, a number of diseases were assigned to immunological causes (immunopathological disorders) and ultimately the subject was offered a separate status. The practice of transfusion of blood from man to man was risky and unpredictable, until the discovery of blood groups by Karl Landsteiner. In recognition of his outstanding work of elucidating the blood groups and Rh factor, he was awarded Nobel Prize in 1930.

The concept of immunocomplexes and their important contribution to immunopathology, came with the development of radioactive tracers and immunofluorescent dyes. With these tracers and dyes, foreign proteins were tagged and followed, after they were injected into the body and their ultimate pathologic consequences were explored. It was impossible to understand the function of the cells in the immune response with the limited techniques available in the early part of the century. Lymphocytes were noted and their importance speculated, but these monotonously similar looking cells were difficult to study. The development of immunofluorescent techniques (Coons and his colleagues, 1942) allowing for the localization of the unique products, the introduction of modern protein chemistry and the use of cell culture techniques helped to a great extent, in understanding the vast complexity of the defense system.

Successions of theories were put forwarded from time to time, to explain the specificity, memory and other features of immune responses. Instructive theories such as direct template theory (Breinl and Haurowitz, 1930; Alexander, 1931; Mudd, 1932) and indirect theories (Burnet and Fenner, 1949) were the subject of criticism by Niels Jerne and Burnet. Burnet's proposition of clonal selection theory was universally accepted as this theory shifted the immunological specificity to the cellular level. The clonal selection theory also explained tolerance as the deletion or suppression of entire clone of cells occurred before or soon after birth. Burnet was awarded Nobel Prize in 1960, which he shared with Peter Medawar.

Most credit went to Rodney R Porter and Gerald M Edelman in elucidating the structure of antibody. Edelman showed that the immunoglobulin molecule had polypeptide chains, two heavy and two light. For their outstanding contribution, they were awarded Nobel Prize in 1972.

The existence of the markers of the biological individuality (histocompatibility antigens) was first suggested by Gore in 1937. Snell, in 1948, showed that the mouse H-2 locus is genetically complex. Dausset identified the human leukocyte antigen (HLA) locus or major histocompatibility complex (MHC). Benacerraf showed that genes of HLA determining loci may control immune response. In 1980, Snell, Dausset and Benacerraf were awarded Nobel Prize for their respective contribution in the field.

Niels Jerne was awarded Nobel Prize in 1984, for his contributions in immunology, the most fundamental of his role in developing the concept of clonality and for his description of the idiotype network in the regulation of immune response.

An ingenious method for large scale production of monoclonal (monospecific) antibody (mAb) against any desired antigen was developed by Georges E Kohler, Cesar Milstein and Niels K Jerne in 1975. They produced a hybrid cell by fusing antibody-forming cell with a myeloma cell. The production of mAb by hybridoma technique created a revolution in the field of immunology in opening up various researches, diagnostic and therapeutic procedures. In recognition of their work, they were awarded Nobel Prize in 1984.

Another, Stalwart (Susumu Tonegawa) was honored by Nobel award in 1987 for his study on genetics of antibody production.

Murray and Thomas were awarded Nobel Prize in the year 1990 for their work 'Use of immunosuppressive drugs in transplantation'. Doherty and Zinkernagel were also honored by Nobel award in 1996 for the work 'Role of MHC in antigen recognition by T cells'. In 2002, Sydney Brenner, Robert H Horvitz and JE Sulston were awarded Nobel Prize for 'Genetic regulation of organ development and cell death (apoptosis).'

The immunology as a subject has grown immensely in the last 100 years. Louis Pasteur, Metchnikoff, Ehrlich, Bordet and many others contributed importantly and critically to the birth and robust development of the science of immunology. The amount of detail knowledge that has been accumulated has already reached paroxysmal proportion and the rate of accumulation, far from abating, is itself increasing. But the most discouraging fact is that the theoretical explosion of knowledge has not been able to make significant contribution to the management of situation as important as allergy, organ transplantation and autoimmune diseases and even, ironically, anti-infectious diseases.

If, there has been some progress that results from the practical aspect of pure scientific knowledge, it is the domain of vaccinology. The control of number of diseases that cause significant mortality and morbidity has made outstanding progress, but there remains a crying need for vaccine against others. Every year millions of death, throughout the world, are caused by tuberculosis, malaria and acquired immune deficiency syndrome (AIDS), the diseases for which there are no effective vaccines. It is hoped that the immunologists of today, using the tools of molecular and cellular biology, genomics, proteomics, will make inroads into preventing these diseases.

**Table 1.1** Nobel Prize winners in the field of immunology

| Year | Name | Discovery |
|---|---|---|
| 1901 | Emil von Behring | Serum antitoxin |
| 1905 | Robert Koch | Cellular immunity to tuberculosis |
| 1908 | Paul Ehrlich | Theories of immunity |
| 1908 | Metchnikoff | Phagocytosis |
| 1913 | Richet | Anaphylaxis |
| 1919 | Jules Bordet | Immunity (work on complement) |
| 1930 | Landsteiner | Blood group |
| 1960 | Burnet and Medawar | Immunological tolerance |
| 1972 | Edelman and Porter | Nature and structure of antibody |
| 1977 | Yalow | Radioimmune assays |
| 1980 | Benacerraf, Snell and Dausset | Major histocompatibility complex (MHC) genes and transplantation |
| 1984 | Milstein, Köhler and Jerne | Monoclonal antibody |
| 1987 | Susumu Tonegawa | Genetics of antibody production |
| 1990 | Murray and Thomas | Use of immunosuppressive drugs in transplantation |
| 1996 | Doherty and Zinkernagel | Role of MHC in antigen recognition of T cells |
| 2002 | Sydney Brenner, H Robert Horvitz and JE Sulston | Genetic regulation of organ development and cell death (apoptosis) |

As it had happened, it is expected that the world would witness further explosion of knowledge in immunology in coming decades. Concerted efforts are needed to be directed in the subjects such as tumor immunology, autoimmunity and vaccinology to develop strategies for protection against many crippling diseases. Eventually what has evolved as a precise and powerful tool created by nature to ensue continued survival of the species, would perhaps be manipulated to yield better quality of life (Table 1.1).

## STUDY QUESTIONS

### Essay Questions

1. Explain the contributions made to the science of immunology over the centuries.

### Short Notes

1. Edward Jenner.
2. Louis Pasteur's contribution in immunology.
3. Charles Richet.
4. Karl Landsteiner.
5. Elie Metchnikoff.

### Match the Scientists with Their Contributions in the Respective Fields

| Scientists | Contributions |
|---|---|
| Robert Koch | Genetics of antibody production |
| Jules Bordet | Radioimmune assay |
| Susumu Tonegawa | Cellular immunity to tuberculosis |
| Yalow | Work on complement |

## SUGGESTED READING

1. Bhatia R, Ichpujani RL. Essential of Medical Microbiology, 4th edition; 2008.
2. Cazenave PA, Talwar GP. Pasteur's Heritage. Willy Eastern Limited; 1991.
3. Goldsby RA, Kindt Thomas J, Osborn Barbara A. Kulay Immunology, 6th edition. New York: WH Freeman and Company; 2007.
4. Paniker A. Textbook of Microbiology, 6th edition; 2000.
5. Parija SC. Textbook of Microbiology and Immunology, 1st edition; 2009.
6. Settee N. Immunology (Introductory Textbook). New Age International (P) Limited; 1994.

# Host-microbe Relationship and Disease Process

# 2

Infection and immunity involve interaction between the host and the infecting microorganism. Based on their relationship to their hosts, microorganisms can be classified as saprophytes (from Greek, sapros—decayed and phyton—plant) and parasites. Saprophytes are free-living microbes that depend on dead and decaying organic matter. They are found in soil and water and play an important role in the degradation of organic materials in nature. Parasites are microbes that can establish themselves and multiply in hosts.

Symbiosis is an association between two (or more) species meaning 'living together'. The term symbiosis includes a spectrum of relationship, they are mutualism, commensalism and parasitism.

Mutualism is a type of symbiosis that benefits both the organisms. For example, the large intestine contains bacteria such as *Escherichia coli* that synthesize vitamin K and some B vitamins. These vitamins are absorbed into the bloodstream and distributed for use by body cells. The bacteria in turn get a favorable environment to obtain nutrients.

In commensalism, one of the organism is benefited and the other is unaffected (neither benefits nor harmed). For example, many microorganisms live on our skin surfaces and utilize the metabolic products secreted from the pores of the skin. Ordinarily we are neither benefited nor harmed. But a harmless commensal could act as a parasite if it gains access to part of the body, where it could not normally exist [*E. coli* causing urinary tract infection (UTI)].

At the other end of the spectrum is the parasitism in which one organism, the parasite benefits from the relationship, whereas the other organism is harmed by it.

## CONTAMINATION, INFECTION AND DISEASE

1. Contamination: It means that the microorganisms are present on inanimate objects, the surface of the skin and mucous membrane.
2. Infection: It is the lodgment and multiplication of the parasite in or on the host tissue.
3. Infestation: It refers to the presence of a large parasite, such as worms or arthropods in or on the body.
4. Disease: It is the disturbance in the state of health, where the body cannot carry out its normal functions.

### Extent of Host Involvement (Characteristic of Infection)

Infections can be classified according to the extent to which the host's body is affected.

*Acute disease:* Disease in which symptoms develop rapidly and that runs its course quickly.

*Chronic disease:* Disease in which symptoms develop slowly and it is slow to disappear.

*Subacute disease:* Disease in which symptoms intermediate between acute and chronic.

*Latent infection:* Disease in which symptoms appear or reappear long after infection.

*Primary infection:* Initial infection with a parasite in a host.

*Secondary infection:* New parasite sets up infection in a host, whose resistance is lowered by a pre-existing infectious disease.

*Reinfections:* Subsequent infections by the same parasite in the host.

*Local infection:* Infection confined to the small region of the body, such as a boil or bladder infection.

*Focal infection:* Infection in a confined region from which pathogens travel to other regions of the body, such as abscessed tooth or infected sinuses.

*Cross infection:* When a patient already suffering from a disease, a new infection is set up from another host or another external source.

*Nosocomial infection:* Cross infections occurring in hospitals.

*Iatrogenic infection:* Physician-induced infections resulting from investigative, therapeutic or other procedures.

*Systemic infection:* Infection in which the pathogen is spread throughout the body, often by traveling through blood or lymph.

*Exogenous infection:* Infection from external sources.

*Endogenous infection:* Infection from hosts own body.

*Inapparent infection:* Infection that fail to produce full set of signs and symptoms.

*Atypical infection:* Infection in which typical or characteristic clinical manifestations of the particular disease are not present.

*Mixed infection:* Infection caused by two or more pathogens.

*Superinfection:* Secondary infection that is usually caused by an agent resistant to the treatment for primary infection.

*Septicemia:* Presence and multiplication of pathogens in the blood leading to manifestations.

*Bacteremia:* Presence, but not multiplication of bacteria in the blood.

*Viremia:* Presence, but not multiplication of viruses in blood.

*Toxemia:* Presence of toxins in the blood.

*Sapremia:* Presence of metabolic products of saprophytes in the blood.

## Source of Infection

### *Human Being*

The commonest source of infection for human being is the human being. Human being gets the infection from either patient or a carrier.

Carrier is a person who harbors the parasite, but himself does not suffer from the illness. There are six types of carriers.

1. Healthy carrier is a person who harbors the pathogen, but has never suffered from the disease.
2. Convalescent carrier is a person who recovered from the disease, but continues to harbor the pathogen.
3. Temporary carrier is a person, who harbors the parasite for less than 6 months.
4. Chronic carrier is a person who harbors the parasite for several months, some times for the rest of one's life.
5. Contact carrier is a person, who acquires the pathogen from a patient.
6. Paradoxical carrier is a person, who gets the pathogen from a carrier.

### *Animals*

Animals may act as source of infection without suffering. Such animals maintain the parasites in nature and acts as reservoir of human infections. The transmission of infections from animals to man are called zoonoses. The zoonotic diseases are bacterial, viral, parasitic and fungal.

*Bacterial:* The diseases caused by bacteria are as follows:

1. Plague from rats.
2. Brucellosis and tuberculosis from cows.
3. Anthrax from sheep.
4. Leptospirosis from dogs, pigs, sheep, cows and rodents.
5. Psittacosis from parrots and other birds.
6. Relapsing fever from rodent (via ticks and lice).
7. Salmonellosis from dogs, cats and poultry.

*Viral:* The diseases caused by virus are as follows:

1. Rabies from dogs.
2. Japanese encephalitis from pigs.
3. Lassa fever, hantavirus infection via rodents.

*Parasitic:* The diseases caused by parasites are as follows:

1. African sleeping sickness from wild games (via tsetse fly).
2. Hydatid disease from dogs.
3. Leishmaniasis from dogs (via sand fly).
4. Tapeworm infection from cattle, swine and rodents.
5. Toxoplasmosis from cats, bats, rodents and domestic animals.

*Fungal:* The diseases caused by fungus are as follows:

1. Histoplasmosis from birds and bats.
2. Ringworm (Tinea) from cats, dogs and other domestic animals.

### *Insects*

Insects may transmit the pathogens to humans. Insects such as mosquitoes, ticks, lice and flies, which transmit the pathogens are known as vectors. Vectors may be:

1. Biological vectors, where pathogens multiply and undergo developmental cycle (malaria, filaria, kala-azar, etc.).
2. Mechanical vectors, which transmit infective parasite mechanically or passively by holding in their appendages, e.g. amebic cysts are carried by housefly from feces to food.

Besides vectors, some insects may also act as reservoir hosts (e.g. ticks in relapsing fever and spotted fever). Infection is maintained in such insects by transovarial passage.

### *Soil and Water*

Some pathogens are able to survive in soil (spores of tetanus and gas gangrene bacilli, *Histoplasma capsulatum*) and water (*Vibrio cholerae,* hepatitis A and E).

### *Food*

Contaminated food material may act as source of infection (food poisoning by *Staphylococcus aureus, Bacillus cereus, E. coli,* etc.). There may be also pre-existing infections of meat, milk, etc. *(Salmonella typhimurium, Mycobacterium tuberculosis).*

## Methods of Transmission of Infection

The methods of transmission of infection (Fig. 2.1) are as follows:

1. Contact transmission.
2. Transmission by vehicles.
3. Transmission by vectors.

### *Contact Transmission*

*Direct contact transmission:* In horizontal transmission, individuals acquire pathogens by shaking hands, kissing, touching or by having sexual contact causes sexually transmitted disease (STD) such as acquired immunodeficiency syndrome (AIDS). Pathogens can also spread by touching genital herpes lesions and then touching the eyes and it also spreads from fecal matter to mouth by unwashed hands (fecal-oral transmission).

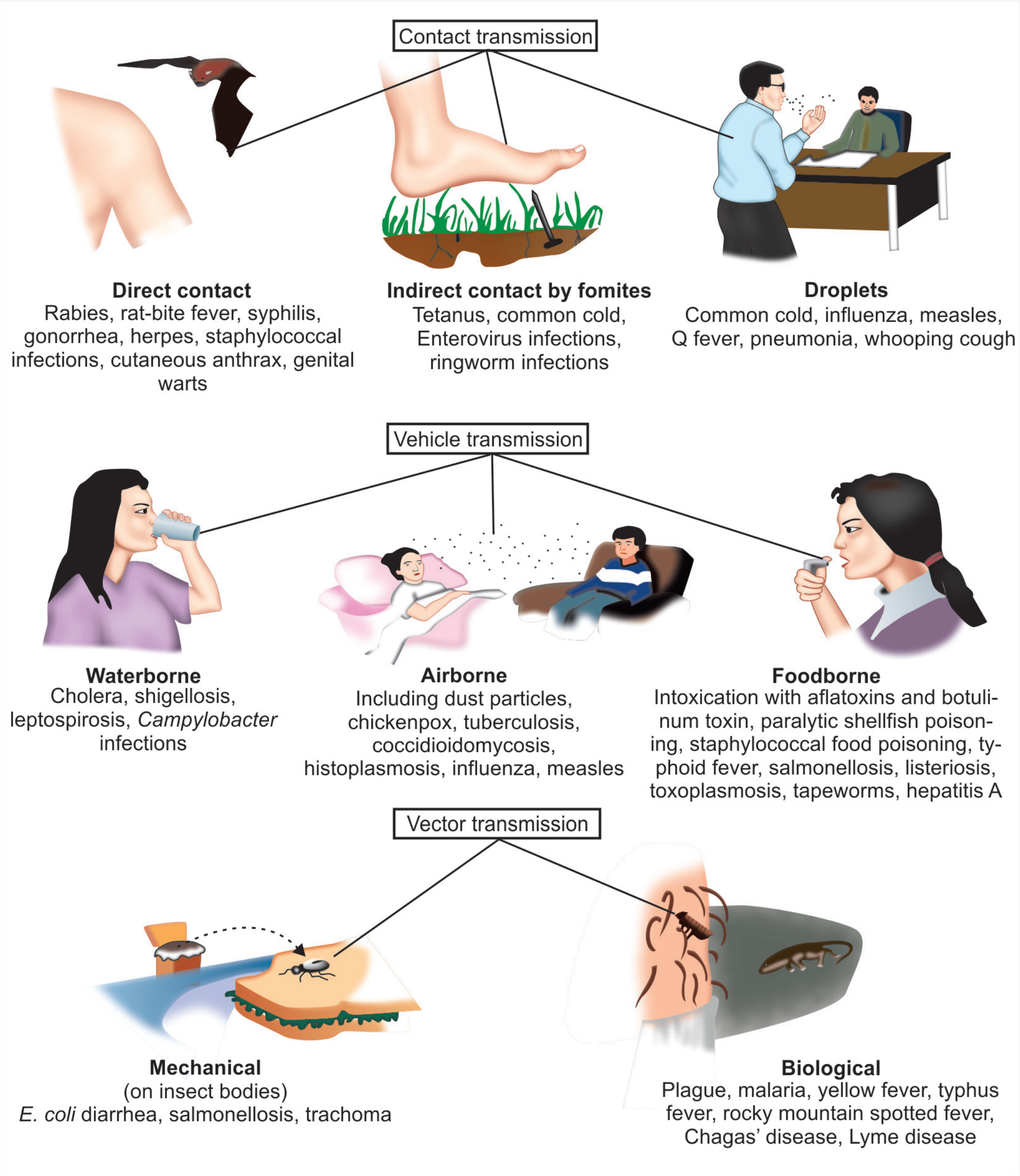

Contact transmission
Direct contact
Rabies, rat-bite fever, syphilis, gonorrhea, herpes, staphylococcal infections, cutaneous anthrax, genital warts
Indirect contact by fomites
Tetanus, common cold, Enterovirus infections, ringworm infections
Droplets
Common cold, influenza, measles, Q fever, pneumonia, whooping cough
Vehicle transmission
Waterborne
Cholera, shigellosis, leptospirosis, *Campylobacter* infections
Airborne
Including dust particles, chickenpox, tuberculosis, coccidioidomycosis, histoplasmosis, influenza, measles
Foodborne
Intoxication with aflatoxins and botulinum toxin, paralytic shellfish poisoning, staphylococcal food poisoning, typhoid fever, salmonellosis, listeriosis, toxoplasmosis, tapeworms, hepatitis A
Vector transmission
Mechanical
(on insect bodies)
*E. coli* diarrhea, salmonellosis, trachoma
Biological
Plague, malaria, yellow fever, typhus fever, rocky mountain spotted fever, Chagas' disease, Lyme disease

**Fig. 2.1:** Overview of mechanism by which diseases can be transmitted

In vertical transmission, pathogens are passed from mother to offspring in an egg or sperm, across the placenta in the breast milk or in the birth canal (congenital, syphilis and gonorrhea).

*Indirect contact transmission:* Occurs through fomites, non-living objects that can harbor and transmit infectious agents.

*Droplet transmission:* It is a type of contact transmission, which occurs when a person coughs, sneezes or speaks near others. Droplet nuclei consist of dried mucus, which protects microorganisms embedded in it. These particles can be inhaled directly or can become airborne. Droplet transmission over a distance of less than 1 m is not considered airborne (chickenpox, tuberculosis, influenza, measles, histoplasmosis).

### *Transmission by Vehicles*

Vehicle is a non-living carrier of an infectious agent from its reservoir to a susceptible host. Common vehicles are water, air and food. Blood and other body fluids, intravenous (IV) fluids can serve as vehicle for transmission.

*Waterborne transmission:* Pathogens do not grow in pure water. Waterborne pathogens are transmitted in water contaminated with untreated or inadequate treated sewage. Such indirect fecal-oral transmission occurs when pathogens from feces enter contaminated water and the sample is taken by another individual. Polioviruses, enteroviruses (hepatitis A and E) and several bacteria (*Vibrio cholerae, Shigella, Leptospira* and *Campylobacter*) are waterborne microbes that infect the digestive system and cause gastroenteritis.

*Airborne transmission:* Airborne microorganisms are mainly transmitted from soil, water, plants or animals. Pathogens are said to be airborne if they travel more than 1 m in air. Both, airborne pathogens and those that are suspended in droplets, have chance of reaching near hosts in a overcrowded locality.

Airborne pathogens fall to the floor and combine with dust particles to become suspended in aerosols. Dust particles can harbor many pathogens (staphylococci, streptococci, bacterial and fungal spores).

Hospitalized patients are at great risk of getting airborne disease firstly, because of the patient's lower resistance and secondly, higher density of population of pathogens in the environment.

*Foodborne transmission:* Pathogens that are most likely transmitted in food are unhygienically cooked or refrigerated poorly. Foodborne pathogens cause gastroenteritis (botulinum toxins, aflatoxin, staphylococcal poisoning, salmonellosis, listeriosis, toxoplasmosis, tapeworm infection, hepatitis A and E).

### *Transmission by Vectors*

Insects act as mechanical vectors. House flies and other insects transmit pathogens passively on their feet and body parts. The pathogens do not multiply in the vector.

Insects act as biological vectors who transmit pathogens actively, i.e. the pathogenic agent must complete part of its life cycle in the vector before the insect can transmit the infective form of the microbe (malaria, filaria, kala-azar, African sleeping sickness, plague, Lyme disease, Chagas' disease).

## MICROBIAL PATHOGENICITY

The term pathogenicity and virulence refers to the ability of a microbe to produce disease or tissue injury, but there is a minor difference between these two.

Pathogenicity is the capacity to produce disease. An organism's pathogenicity depends on its ability to invade a host, multiply in the hosts. *Mycobacterium tuberculosis* frequently causes disease upon entering the host, where as *Staphylococcus epidermidis* cause disease rarely, when it enters into a host with poor defense. Most infectious agents exhibit a degree of pathogenicity between these extremes.

Virulence is the degree of pathogenicity. This term also refers to the intensity of the disease produced by pathogens and varies among different microbial species. For example, *Bacillus cereus* causes mild gastroenteritis, where as rabies virus causes fatal neurological damages. Virulence also varies among members of main species. Organisms freshly discharged from an infected individual tend to be more virulent than those from a carrier. The virulence of a strain is not constant and may undergo spontaneous or induced variation. Enhancement of virulence is known as exaltation, which can be done by serial passage, the rapid transfer of the pathogen through susceptible animals several times. Presumably the microbe becomes better able to damage the host with each animal passage. Reduction of virulence is known as attenuation, which can be achieved by:

1. Passage through unfavorable host.
2. Repeated culture in artificial media.
3. Growth under high temperature.
4. Growth in the presence of week antiseptic solution.
5. Desiccation.
6. Prolonged storage in culture.

Virulence is the sum total of several determinants as detailed below.

## Microbial Virulence Factors

Virulence factors are structural or physiological characteristics that help the microorganisms to cause infection and disease. Microbial virulence factors are as follows:

- Adhesion
- Invasiveness
- Toxigenicity
- Enzymes and other bacterial products
- Inhibition of phagocytosis
    - Inhibition of chemotaxis
    - Inhibition of attachment of phagocytes
    - Inhibition of lysosome fusion
    - Resistance to killing in phagolysosomes
    - Escape from phagolysosomes into cytoplasm
- Escape from host immune response
    - By antigenic variation
    - Superantigen
    - Reduction in expression of antigen
    - Immunosuppression
    - Antibody cleavage
    - Serum resistance
    - Induction of ineffective (blocking) antibodies
- Acquisition of nutrients from the host.

### *Adhesion*

Once the bacteria enter the body of the host, the critical point in the production of bacterial disease is the organisms adherence or attachment to the host cell surface. If they did not adhere, they would be swept away by the mucus and other fluids that bathe the tissue surface. Adhesins are proteins or glycoproteins found on pili (fimbriae) and capsules. Most adhesins that have been identified permit the pathogens to adhere only to receptors on certain host epithelial cells (Table 2.1).

Adherence to host epithelial cells may be the essential first step in the pathogenesis of many infectious diseases. Adherence allows the organisms to colonize and multiply at a rate faster than their removal, offers access to body tissues and cells and provides focus for elaboration of enzymes and toxins. Adherence may also be nutritionally important.

The interaction between bacteria and tissue cell surfaces in the adhesion process are complex. Several factors play important roles (Figs 2.2A to C).

1. Surface hydrophobicity and net surface charge: Bacteria and host cells, commonly have a negative surface charges and therefore, have a repulsive electrostatic forces. In general, more hydrophobic the bacterial cell surface, the greater adherence to the host cell.
2. Binding molecules on bacteria (ligands) and host cell receptor interactions also play important role in adhesion.

### *Invasiveness*

Invasion is the term commonly used to describe the entry of the bacteria into the host cells, implying an active role of the organism. For many disease causing bacteria, in-

**Table 2.1** Examples of bacterial adherence mechanism

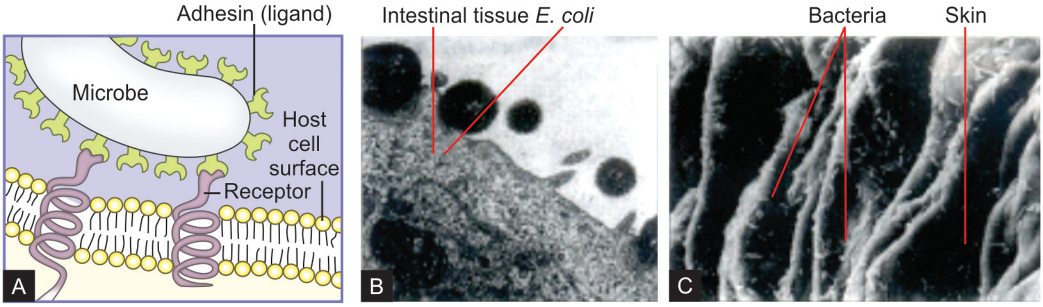
| Microorganisms | Adhesin (ligand) | Receptor |
|---|---|---|
| *Staphylococcus aureus* | Lipoteichoic acid | Unknown |
| *Staphylococcus* species | Slime | Unknown |
| Group A streptococci | Lipoteichoic acid—M protein complex | Fibronectin |
| *Streptococcus pneumoniae* | Protein | N-acetylhexosamine-gal |
| *Escherichia coli* | Type 1 fimbrial colonization factor; Antigen 1 fimbriae: P fimbriae | D-mannose<br>GM ganglioside<br>P blood group glycolipid |
| Other enterobacteriaceae | Type 1 fimbriae | D-mannose |
| *Neisseria gonorrhoeae* | Fimbriae | GD1 ganglioside |
| *Treponema pallidum* | P1, P2, P3 | Fibronectin |
| *Chlamydia* species | Cell surface lectin | N-acetylglucosamine |
| *Mycoplasma pneumoniae* | Protein P1 | Sialic acid |
| *Vibrio cholerae* | Type IV pili | Fructose and mannose |

vasion of the host epithelium is central to the infectious process. Some bacteria such as pneumococci and streptococci release digestive enzymes that allow them to invade tissues rapidly and cause severe illness.

The bacteria also make contact with membrane junction that form part of transport network between host cells. The bacteria use a glycoprotein called cadherin, which bridges the junction to move from cell to cell. The examples of bacterial invasion have been noted in Table 2.2.

### Toxigenicity

Toxins produced by bacteria are generally classified into two groups, exotoxins and endotoxins.



**Figs 2.2A to C**: Interaction between bacteria and tissue cell surface in the adhesion process. **A.** Surface molecules on a pathogen, called adhesins or ligands, bind specifically to complementary surface receptors; **B.** Selective attachment of a pathogenic strain of *E. coli* to the intestinal tissue of a rabbit; **C.** Bacteria adhering to the skin of a salamander.

**Table 2.2** Examples of bacterial invasion

| Microorganisms | Invasion |
|---|---|
| *Salmonella* species | Invade tissues through the junctions between epithelial cells |
| *Yersinia* species<br>*Neisseria gonorrhoeae*<br>*Chlamydia trachomatis* | Invade specific types of host epithelial cells subsequently enter the tissue.<br>Opacity-associated proteins (Oap) enhance the invasion of the cells by *Neisseria gonorrhoeae.* |
| *Shigella* species | Invasion plasmid antigen IpaB, IpaC, IpaD |
| *Listeria monocytogenes* | Invade with the help of internalin protein, through coiling phagocytosis |

### Exotoxins

Exotoxins are heat-labile proteins, which are secreted by certain species of bacteria and diffuse readily into the surrounding medium (Figs 2.3A and B). They are highly potent in minute amount, e.g. 1 mg of botulinum toxin can kill more than million guinea pigs. It has been estimated that 3 kg of botulinum toxin is sufficient enough to kill all the inhabitants of the world. Exotoxins are classified in Box 2.1.

The toxin molecule is secreted as a single polypeptide molecule (62,000 MW). This native toxin is enzymatically divided into fragments, A and B. Fragment ‘B’ (47,000 MW) binds to specific host cell receptor and facilitate the entry of fragment ‘A’ (21,150 MW). Exotoxins are highly antigenic and can be toxoided by treatment with formaldehyde (Fig. 2.4).

**Box 2.1:** Classification of exotoxins

A. Based on site of action:

1. Cytotoxins, e.g. hemolysins, leukocidin.
2. Enterotoxins, e.g. the cholera toxin, enterotoxigenic *Escherichia coli* (ETEC) toxin, enterotoxins of *Staphylococcus aureus.*
3. Neurotoxins, e.g. tetanus toxin, botulinum toxin.
4. Cutaneous exotoxins, e.g. toxin of *Staphylococcus aureus.*
5. Pyrogenic exotoxin, e.g. erythrogenic toxin of *Streptococcus pyogenes,* TSST of *Staphylococcus aureus.*
6. Special toxins, e.g. anthrax toxin, diphtheria toxin.

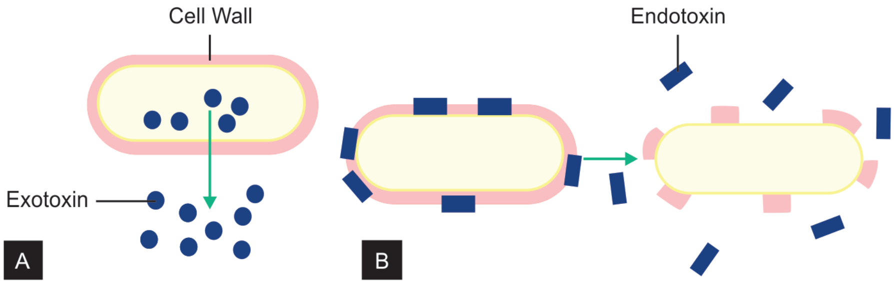
B. Based on mechanism of action of exotoxins:

1. Damage to the cell membrane, e.g. cytotoxins.
2. Alteration of cell function, e.g. cholera toxin, ETEC.
3. Lethal action, e.g. diphtheria toxin (prevents protein synthesis by acting on elongation factor).

### Endotoxins

Endotoxins are heat stable, polysaccharide-protein-lipid complex, which form an integral part of the cell wall of gram-negative bacteria. Their toxicity depends upon the lipid fraction (lipid ′A′). They are not secreted into the medium, weakly antigenic, hence cannot be toxoided. All endotoxins, whether from pathogenic or non-pathogenic have similar effects (Table 2.3).

Endotoxins exert their effects, when gram-negative bacteria die and their cell walls undergo lysis, thus liberating the endotoxins. Antibiotics used to treat diseases caused by gram-negative bacteria can lyse the bacterial cells; this reaction releases endotoxin and may lead to an immediate worsening of the symptoms, but the condition usually improves as the endotoxin breaks down.



**Figs 2.3A and B:** Exotoxin and endotoxin. **A.** Exotoxins are produced inside, mostly in gram-positive bacteria as part of their growth and metabolism. They are then released into the surrounding medium; **B.** Endotoxins are part of the outer portion of the cell wall of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

**Table 2.3** Difference between exotoxins and endotoxins

| Properties | Exotoxins | Endotoxins |
|---|---|---|
| Chemical nature | Protein or short peptide | Lipid A portion of lipopolysaccharides (LPS) |
| Heat stability | Unstable, denatured above 60°C and by ultraviolet | Relatively stable and can with stand several hours above 60°C |
| Location in the cell | Extracellular excreted into the medium | Bound within the bacterial cell wall, released upon death of bacterium |
| Extraction | Can be separated by filtration | Obtained only by cell lysis |
| Pharmacology (effects on body) | Specific pharmacological action on each toxin | Non-specific, common to all endotoxin |
| Tissue affinity | Specific tissue affinity | No tissue affinity |
| Toxicity lethal dose (ability to cause disease) | High, active in very minute doses | Low, active in large doses |
| Immunogenicity and toxoid conversion | Highly antigenic and can be toxoided | Weakly antigenic can not be toxoided |
| Bacterial source | Mostly from gram-positive bacteria. Some gram-negative bacteria *Vibrio cholerae,* enterotoxigenic *Escherichia coli* (ETEC), etc. | Gram-negative bacteria |
| Binding site | Binds to specific receptors on cells | Specific receptors not found on the cell |
| Fever production | Little or no fever | Produce high temperature by release of interleukin-1(IL-1) |
| Control | Frequently controlled by extra chromosomal genes (plasmids) | Controlled by chromosomal genes |
| Examples | Botulism, gas gangrene, tetanus, diphtheria, staphylococcal, food poisoning, cholera, enterotoxin, plague | Salmonellosis, tularemia, meningococcemia, endotoxic shock |

Responses by the hosts to endotoxin include chills, fever, weakness, generalized ache and in some cases, shock and even death. Another consequence of endotoxin is the activation of clotting factor (Hageman factor) causing formation of small blood clots. These blood clots obstruct capillaries. At the same time lipopolysaccharide acts on plasminogen, producing plasmin, which acts on clots and breakdown fibrin clot. There is reduction in fibrinogen, accumulation of fibrin breakdown products that brings about disseminated intravascular coagulation.

Pyrogenicity of endotoxin is believed to occur when gram-negative bacteria are ingested by phagocytes and degraded in the vacuoles, the lipopolysaccharides (LPS) of the bacterial cell wall is released. These endotoxins activate macrophage to produce interleukin-1(IL-1), endogenous pyrogen,

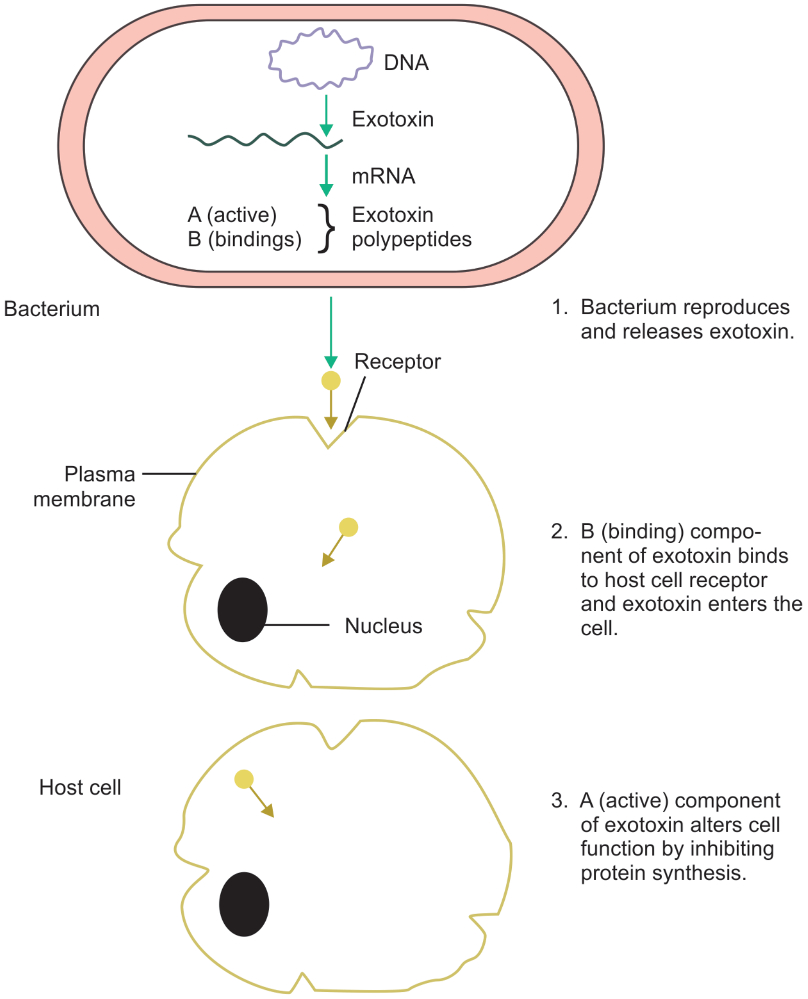

DNA
Exotoxin
mRNA
A (active)
B (bindings)
Exotoxin
polypeptides
Bacterium
Receptor
Plasma
membrane
Nucleus
Host cell
1. Bacterium reproduces and releases exotoxin.
2. B (binding) component of exotoxin binds to host cell receptor and exotoxin enters the cell.
3. A (active) component of exotoxin alters cell function by inhibiting protein synthesis.

**Fig. 2.4:** The action of an exotoxin. A proposed model for the mechanism of action of diphtheria toxin (DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid).

which are carried via blood to the hypothalamus, a thermoregulatory center in the brain. IL-1 induces the hypothalamus to release lipids called prostaglandins, which reset the thermostat in the hypothalamus at higher temperature. The result is fever. Aspirin and acetaminophen reduces fever by inhibiting the synthesis of prostaglandins (Figs 2.5A to D).

Shock refers to life-threatening fall of blood pressure. Shock caused by gram-negative bacteria is called septic shock or endotoxic shock. Phagocytosis of gram-negative bacteria cause the phagocytes to secrete a polypeptide called tumor necrosis factor-alpha (TNF-α) or cachectin. TNF-α binds to the tissue and alters their metabolism in a number of ways. One effect of TNF-α is damage to blood capillaries, their permeability is increased and they lose large amounts of fluid. Hence there is a drop in blood pressure resulting in shock.

*Limulus amebocyte lysate (LAL) assay:* This is a highly sensitive laboratory test, which can detect even minute amount of endotoxin (10 –20 pg of endotoxin/mL) in drugs, medical devices, body fluids and in IV fluids.

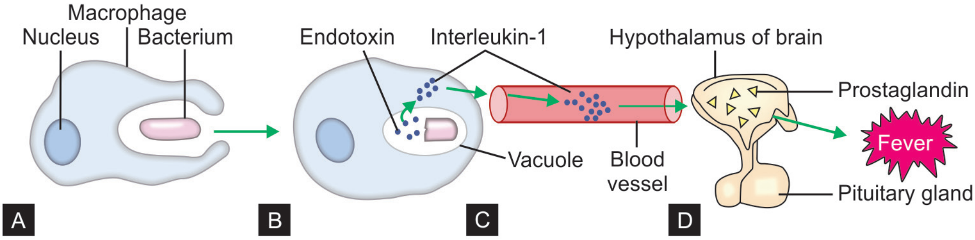
The hemolymph (blood) of the Atlantic coast horseshoe crab *(Limulus polyphemus)* contains white blood cells (WBCs) called amebocytes, which have large amount of protein (lysate) that cause clotting. In the presence of endotoxin, amebocytes in the crab hemolymph lyse and liberate their clotting protein. As a result there will be clot formation in positive test.

*Rabbit pyrogenicity test:* This test is done on rabbit, a pyrogen sensitive mammal to know the pyrogenic effect of endotoxin. The sample of solution suspected to be contaminated with LPS of gram-negative bacilli is injected intravenously into the ear veins of adult rabbits. The rectal temperature of the animal is monitored before and after the IV injection of solution. If the solution contains endotoxin, the rectal temperature of the animal will be higher than normal indicating the pyrogenic effect of endotoxin.

### *Enzymes and Other Bacterial Products*

The virulence of some bacteria is thought to be aided by the production of enzymes, hemolysin, cytocidin, etc.

Coagulase is an enzyme produced by *S. aureus,* which helps in conjunction with serum factors to coagulate plasma. Coagulase helps in deposition of fibrin on the surface of *Staphylococcus,* which protect them from being phagocytosed.



**Figs 2.5A to D**: Pyrogenicity of endotoxin. **A.** A macrophage ingests a gram-negative bacterium; **B.** The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce interleukins (IL-1); **C.** IL-1 is released by the macrophage into the bloodstream through which it travels to the hypothalamus of the brain; **D.** IL-1 induces the hypothalamus to produce prostaglandins, which reset the body's 'thermostat' to a higher temperature, producing fever.

Hyaluronidase (spreading factor) of *Streptococcus pyogenes* hydrolyze hyaluronic acid, the ground substance and helps in spreading the lesions.

Collagenase and lecithinase produced by *Clostridium perfringens* facilitates the spread of gas gangrene.

Hemolysins and leukocidins of *S. aureus* dissolve red blood cells (RBCs) and kill leukocytes respectively. Other hemolysin producers are *Streptococcus pyogenes* and *Clostridium perfringens*. One of the better known kinase is fibrinolysin (streptokinase), which breaks down fibrin and dissolves the clot formed by the body to help in spreading infection. Immunoglobulin ($IgA_1$) proteases (produced by *Neisseria gonorrhoeae, N. meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae*) split IgA at specific site and inactivate its antibody activity.

Other bacterial substances contribute to the virulence are necrotizing factors, which cause the death of the body cells; hypothermic factors, which decrease body temperature; and siderophores, which scavenge iron from host's body fluid for bacterial iron requirement.

### Inhibition of Phagocytosis

Microorganisms have managed to develop a mechanism for inhibiting every step in the normal phagocytic process (Figs 2.6A and B).

*Inhibition of chemotaxis*

Several bacterial toxins are able to inhibit the migration of leukocyte towards chemoattractants (e.g. Cholera toxin of *Vibrio cholerae,* enterotoxin of *E. coli,* streptolysin 'O' of *S. pyogenes*).

Some microbial surface components such as the hyaluronic acid capsule of *S. pyogenes* may also inhibit the locomotion of leukocytes.

*Inhibition of attachment of phagocytes*

The bacterial capsule is by far the most important ubiquitous antiphagocytic substance (e.g. *S. pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *N. meningitidis*).

The mechanism by which encapsulation confers resistance to phagocytosis may include decreased binding of serum opsonins; inaccessibility of ligands (IgG and C3b) required for phagocytic binding and decreased hydrophobicity of the bacterial surface. It should be noted that the presence of anticapsular antibody overcomes the antiphagocytic effect of the capsules.

Numerous other structural components are antiphagocytics. Examples include the protein A of *S. aureus,* the M protein of *S. pyogenes,* the virulence (VI) antigen of *S. typhi* and outer membrane proteins of *N. gonorrhoea*.

*Inhibition of lysosome fusion*

Microorganisms can escape from phagocytosis by inhibiting the fusion of the lysosome with the phagosome (e.g. *Legionella pneumophila, Chlamydia psittaci, Toxoplasma gondii, M. tuberculosis)*.

*Resistance to killing in a phagolysosome*

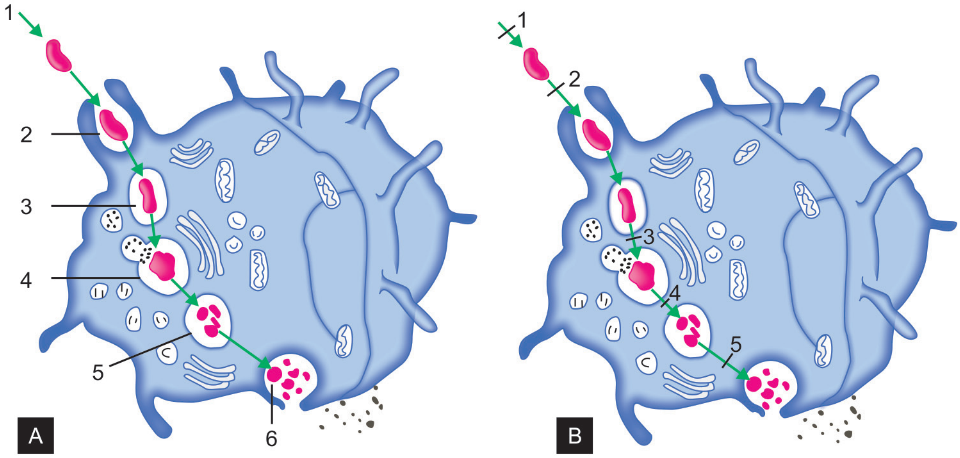
Facultative or obligate intracellular parasites must be able to resist killing by developing the ability to resist either the oxygen-dependent or oxygen-independent antimicrobicidal actions of the phagolysosome enzymes.

*Escape from phagolysosome into cytoplasm:* Some parasites can lyse the phagolysosomal membrane and escape into the cytoplasm.

### Escape from Host Immune Response

*By antigenic variation*

Organisms may evade the immune response by modifying their surface antigens. The best example of antigenic variation is that of African trypanosomiasis. This disease is characterized by repeated episodes of bloodstream invasion and remission. The trypanosomes are able to circumvent the effects of these antibodies by changing the antigens present on their surface coat. Antigenic variation is also



**Figs 2.6A and B**: Phagocytosis of bacteria. **A**. Schematic diagram of the steps in phagocytosis (**1**. Chemotaxis, **2**. Attachment of a bacterium (red) to long membrane evaginations called pseudopodia, **3**. Ingestion of bacterium, forms a phagosome, which moves towards a lysosome, **4**. Fusion of the phagosome and lysosome, releases lysosomal enzymes into the phagosome, **5**. Digestion of ingested material, **6**. Release of digestion products from the cell); **B**. Steps in inhibition of phagocytosis (**1**. Inhibition of chemotaxis, **2**. Inhibition of attachment, **3**. Inhibition of phagolysosomal fusion, **4**. Escape from intracellular killing mechanisms, **5**. Escape from phagolysosome into cytoplasm).

seen in malaria as these parasites go through several developmental stages.

The incorporation of host antigens into its surface membrane may help to avoid the effect of immune response, e.g. *Schistosoma mansoni*.

Viruses may also demonstrate antigenic variation. Influenza virus undergoes minor and major antigenic changes in some of its surface antigens. The antigenic variation of human immunodeficiency virus (HIV) plays a role in the ability of this virus to evade the host immune response. Antigenic variation is seen in *Borrelia* species among bacteria.

*Superantigens*

Superantigens are unique and they bind to specific sites in the variable portion of the chain regardless of the specificity for the antigen. Thus, they are able to stimulate all subsets of T lymphocytes that share these specific Vβ sequences. Furthermore, superantigens do not have to be processed by antigen-presenting cells (APCs). They are able to bind directly to the class II major histocompatibility complex (MHC) antigens and then to the Vβ T cell receptors.

*Reduction in expression of antigens*

Reduction in expression of antigens has been associated with persistent viral infections. Paramyxoviruses, arenaviruses, retroviruses and rhabdoviruses are able to reduce the expression of viral coat glycoproteins. Adenoviruses can also inhibit MHC expression by infected cells.

*Immunosuppression*

A state of immunosuppression may develop in the host during infection with bacteria, viruses, fungi, protozoa and helminths. This immunosuppression may be specific or general, unrelated to the infecting organism. The best example for general immunosuppression is AIDS, the infection of T helper cluster of differentiation (CD4) cells and APCs by HIV results in serious loss of immune function.

General and specific immunosuppression may involve both the humoral and cell-mediated responses.

*Antibody cleavage*

Some microorganisms produce protease enzymes that cleave human IgA. IgA is the principal mediator of humoral immunity at mucosal surfaces.

*Serum resistance*

Serum resistance is the ability of a microorganism to prevent lysis by complement. For example, certain strains of *Salmonella* and *E. coli* possess 'O' antigens in their LPS, which sterically hinder the access of the C5b through C9 (C5b-9) complex, the lytic complex of the complement.

### *Induction of the Ineffective Antibodies*

Ineffective blocking antibodies may be formed in response to certain strains of *N. gonorrhoeae*. These antibodies are able to bind specific receptors on the surface of gonococci and there by block the access to the antigen by the effective antibacterial antibody.

### *Acquisition of Nutrients from the Host*

Bacteria require iron for the metabolism and growth and for production of a variety of toxins. One of the mechanisms that bacteria have developed to extract iron from the host is the production of siderophores. Siderophores, produced by bacteria in the absence of iron are able to extract iron bound to transferrin or lactoferrin and deliver it to the bacterial cell via special receptors.

Much variation exists among the siderophores that have been characterized, but most fall into two categories: catechols (phenolates) of which enterobactin is the best characterized and hydroxamates of which ferrichrome is the best characterized. Enterobactin is produced by *E. coli* and some other enterobacteriaceae. Hydroxamates are commonly found in fungi. Siderophore production is genetically responsive to the concentration of iron in the medium. For example, enterobactin is produced only under low iron conditions. Enterobactin can remove iron from transferrin, some bacteria do not have demonstrable siderophores. *Yersinia pestis* can utilize iron from hemin and may be able to initiate infection using iron from hemin in gut of the biting flea. *N. gonorrhoeae* makes a series of iron regulated outer membrane proteins. Other bacteria (e.g. *Legionella pneumophila, Listeria* species, *Salmonella* species) can obtain iron from the host intracellular iron pools. The availability affects the virulence of pathogen. For example, the virulence of *N. meningitidis* for mice is increased 1,000 fold or more, when bacteria are grown under iron-limited conditions.

## Role of Bacterial Biofilm

A biofilm is a congregation of interactive bacteria adsorbed to a solid surface or each other and encased by a exopolysaccharide matrix. Biofilm form a slimy coat on solid surfaces (prosthetics such as catheter, heart valves, contact lenses, etc.) and occurs throughout the nature. A biofilm may involve single species bacterium or more than one species. Sometimes fungi, both yeasts and mycelia may form biofilm.

The first step in biofilm formation is colonization of bacteria on the surface, which is facilitated by flagella, fimbriae and some times cell divisions. Bacteria continuously secrete low level molecules called quorum-sensing signals (e.g. echo moresin lactone signals), some bacteria such as *Pseudomonas aeruginosa* produces extracellular alginate.

The organisms, which form biofilm cause persistent infections and pose several problems in the treatment. The common biofilm producing bacteria include *S. epidermidis, S. aureus*, *P. aeruginosa,* etc. *Staphylococcus epidermidis* and *S. aureus* cause infections of central venous catheters, infections of eye colonizing on contact lenses and intraocular lenses. There are many other examples also.

The bacteria in the exopolysaccharide matrix are protected from the host's immune mechanisms. Because of the matrix there is a diffusion barrier, which does not allow the antimicrobials to reach the organisms there by developing resistance.

## Regulation of Virulence Factors

Virulence factors in microorganisms are regulated by genes or environmental factors. Virulence factors are coded by genes, which may be present on chromosomes, plasmids, transposons and bacteriophages. Environmental factors, which regulate virulence of microorganisms include temperature, pH, osmotic pressure and iron concentrations.

Plasmids are small, circular extrachromosomal deoxyribonucleic acid (DNA) molecules. Some plasmids called R-factor (resistance) are responsible for the resistance of some microorganisms to antibiotics. In addition, a plasmid may carry the information that determines a microbe pathogenicity. The examples of virulence factors that are encoded by plasmid genes are tetanospasmin, heat-labile enterotoxins and staphylococcal enterotoxins.

Bacteriophages can incorporate their DNA into the bacterial chromosome, becoming a prophage and thus remain latent and do not cause lysis of the bacteria. Such a state is called lysogeny and cells containing the prophage are said to be lysogenic. One outcome of lysogeny is that the host bacterial cell and its progeny acquire new properties coded for the bacteriophage. Such a change in the characteristics of a microbe due to prophage is called lysogenic conversion. Among bacteriophage genes that contribute to pathogenicity are the genes for diphtheria toxin, erythrogenic toxin, staphylococcal enterotoxin, botulinum toxin and the capsule produced by *Streptococcus pneumoniae.*

### *Virulence Factors in Viruses*

Viruses can replicate only in host cells, where the components of the immune system cannot reach them. Viruses gain access to the cells, because they have got attachment sites for receptors on their target cells. Some viruses gain access to the host cells because their attachment sites mimic substances useful to those cells. For example, the attachment sites of rabies virus can mimic the neurotransmitter acetylcholine. As a result the virus can enter the host cell along with the neurotransmitter.

In many viruses, additional proteins are produced that interfere at various levels with specific and non-specific defenses. The advantage of having such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses. Virus such as hepatitis C evolves the strategies to evade the action of interferon (IFN)-$\alpha$ and IFN-$\beta$ by blocking the action of protein kinase receptor [Refer to Chapter 18 (Fig. 18.3)]. Both herpes simplex virus (HSV-1) and HSV-2 express an immediate early protein called infected cell protein (ICP) 47, which very effectively inhibit the human transporter with antigen processing (TAP). The targeting on MHC molecule, in addition to HSV, also occurs in adenovirus and cytomegalovirus (CMV), which lead to down regulation of MHC I expression inhibiting antigen presentation to CD8 T lymphocytes.

Many other viruses maintain their virulence, evading the immune defense by adopting antigenic variation. The best example is influenza virus, which undergoes antigenic variation producing a different strain against which the existing antibody becomes ineffective. Antigenic variation also occurs in HIV due to regular mutation, ultimately evading the defense mechanism, causing drug resistance and creating impediments in preparation of vaccines.

Some viruses directly destroy the lymphocytes and macrophages (HIV causing lysis of CD4 T cells) causing profound decrease in cell-mediated immunity.

Viruses can bring about cell responses leading to lysis of cell or there may be inclusion body formation or cell dysfunction. Retroviruses and oncogenic viruses integrate into the host chromosomes and can remain in cells indefinitely, sometimes leading to the expression of there antigens on the host surfaces.

### *Virulence Factors in Fungi*

Fungi damage host tissues by releasing enzymes that attack cells. Some fungi produce toxins or cause allergic reaction in the host. Certain fungi produce mycotoxins, which cause disease if ingested by human being (e.g. ergot, aflatoxin).

*Cryptococcus neoformans* possesses a polysaccharide capsule, which inhibits phagocytosis, though this can be overcome by opsonization with complement and antibodies. *Histoplasma capsulatum,* an obligate intracellular fungus, evades macrophage killing by entering the cell via complement receptor (CR3) and then altering the normal pathways of phagosome maturation. Dermatophytes suppress host T cell responses to delay cell-mediated destruction.

### *Virulence Factors in Protozoa and Helminths*

Pathogenic protozoa and helminths cause disease in several ways. Plasmodial and leishmanial species avoid antibody-mediated destruction by their intracellular existence. CD8 cells cannot affect blood stage malaria parasites, as the erythrocytes do not possess MHC I molecule on their surface. In *Giardia intestinalis,* the virulence factor is an adhesive disc by which it attaches to the cell lining epithelium of intestine and burrow into the tissue. Some parasites (e.g. *Entamoeba histolytica*) are protected by changing to the cystic forms.

Most helminths are extracellular parasites inhabiting the intestine or body tissues. Many release toxic waste products and an-

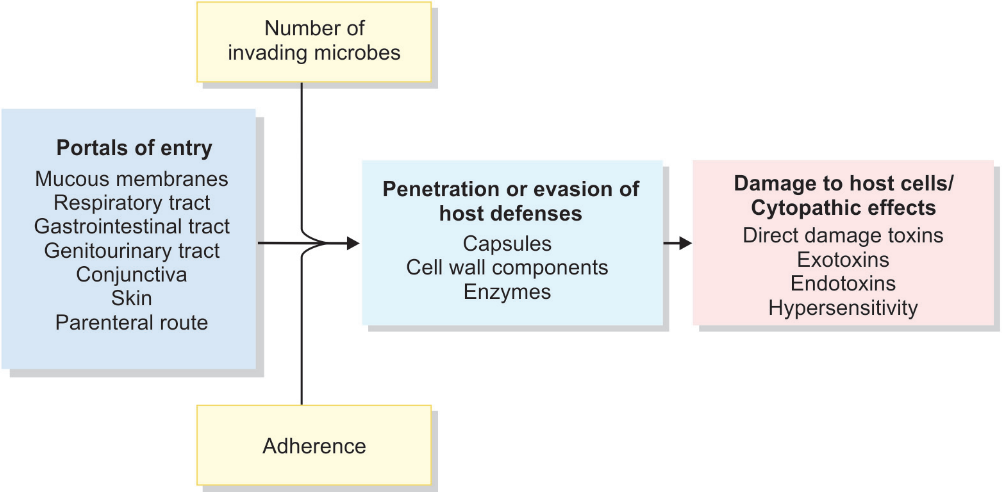

Number of invading microbes
Portals of entry
Mucous membranes
Respiratory tract
Gastrointestinal tract
Genitourinary tract
Conjunctiva
Skin
Parenteral route
Penetration or evasion of host defenses
Capsules
Cell wall components
Enzymes
Damage to host cells/ Cytopathic effects
Direct damage toxins
Exotoxins
Endotoxins
Hypersensitivity
Adherence

**Fig. 2.7:** The overall scheme of microbial pathogenicity

tigens in their excretions (e.g. ascarase by female roundworms) and cause allergic reactions in the host. Roundworms cause obstruction in the intestine or in extraintestinal sites (Fig. 2.7). *Trichinella spiralis* can cause disruption of tissue directly producing a soluble lymphotoxic factor. *Schistosoma* species acquire a surface layer of host antigens, so that the host defense does not distinguish them from the self. Schistosomas can cleave a peptide from IgG. The soluble helminthic parasite antigens may reduce the effectiveness of host response by a process called immune distraction.

## STUDY QUESTIONS

### Essay Questions

1. Define infection and infectious disease. Discuss about the microbial virulence factors.
2. Enumerate the microbial virulence factors. Explain the mechanism of action of endotoxins.
3. How do you explain host parasite relationship in commensalism, parasitism? What are the factors favoring parasitism?

### Short Notes

1. Exotoxin.
2. Endotoxin.
3. Differences between exotoxin and endotoxin.
4. Toxoid.
5. Definitive host.
6. Intermediate host.
7. Carriers—various types.
8. Vector.
9. Zoonotic diseases.
10. Virulence.
11. Congenital infection.
12. Bacterial biofilm.

## SUGGESTED READING

1. Black JG. Microbiology Principles and Applications, 3rd edition. USA: Printice Hall College Div; 1996.
2. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
3. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
4. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
5. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
6. Litwin CM, Claderwood SB. Role of iron in regulation of virulence genes. Clin Microbiol Reviews; 1991;6:137-49.
7. Mims EA. The Pathogenesis of Infectious Disease, 3rd edition. London: Academic press; 1987.
8. Paniker A. Textbook of Microbiology, 8th edition; 2009.
9. Patrick S, Larkin MJ. Immunological and Molecular Aspects of Bacterial Virulence. Wiley Chichester; 1995.
10. Peakman M, Vergain D. Basic and Clinical Immunology, 1st edition; 1997.
11. Poxton IR, Arbuthnot JP. Determinants of bacterial virulence, chap 13. Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8th edition, Vol I. London: Edward Arnold; 1990.
12. Roth JA, et al. Virulence of mechanisms of bacterial pathogens. Washington DC: American Society for Microbiology; 1998.
13. Salmond GPC, Bycroft BW, Stewart GSAB, et al. Molecular biology; 1995;16:615-24.
14. Schaechter M, Cary N, Barr I. Mechanisms of Microbial Disease, 3rd edition; 1998.
15. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's Illustrated Reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
16. Torotra, Funke, Case. Microbiology an Introduction, 6th edition; 1998.

# Immunity

# 3

Pathogenic microorganisms are endowed with special properties that enable them to cause disease, if given the right opportunity. If microorganisms never encounter resistance from the host, we would constantly be ill and would eventually die of various diseases. But in most cases, our body defenses prevent them of happening so. In some instances, the body does not allow the organisms to enter. In others, even if they enter, are eliminated by different mechanisms. In still others, even if they remain inside, the defenses combat with them. Our ability to ward off disease in general is called resistance (immunity). Vulnerability or lack of resistance is susceptibility.

Immunity is defined as the resistance exhibited by the host towards injury caused by the microorganisms and their products.

Protection against the infectious agents is only one of the consequences of the immune response. But in true sense, immunity involves the defensive response, when a host is invaded by foreign organisms or other foreign substances (pollen, insect venom, transplanted tissue). Body cells that become cancerous are also recognized as foreign and may be eliminated.

## TYPES OF IMMUNITY

Immunity to infectious agents can result from innate immunity, acquired immunity or both (Fig. 3.1).

### Innate Immunity

Innate immunity is an invariable, hereditary response—an inborn defense. It is independent of previous exposure to disease causing agents and foreign substances. The innate immunity depends on the non-specific mechanisms, molecular defenses and the activity of the phagocytic cells. Innate immunity may be non-specific, when it indicates a degree of resistance to infection in general or specific, when resistance to particular pathogen is concerned.

Innate immunity may be considered at the level of species, race and individual. In species immunity, all individuals of a species are born with resistance to an infectious agent that causes disease in another species. For example, humans are immune to most infectious agents that causes disease in pets and other domesticated animals. Human beings are insusceptible to rinderpest or distemper, which the canines suffer. Similarly, the animals show innate immunity to many human pathogens. The mechanisms of species immunity are not clearly understood, but may be due to physiological and biochemical differences between the tissues of the different host species that determine, whether or not a pathogen can multiply in them.

Within a species, different races show difference in susceptibility to infections. This is known as racial immunity. The classical

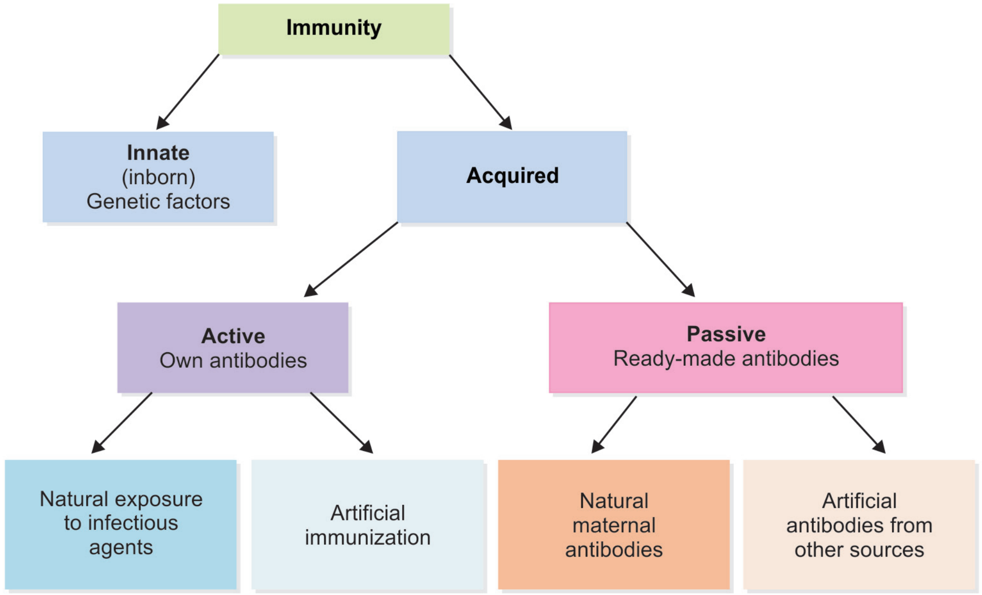

Immunity
Innate
(inborn)
Genetic factors
Acquired
Active
Own antibodies
Passive
Ready-made antibodies
Natural exposure to infectious agents
Artificial immunization
Natural maternal antibodies
Artificial antibodies from other sources

**Fig. 3.1:** Various types of immunity. Non-specific immunity is largely innate or inborn, whereas specific immunity is acquired.

example of racial immunity is the resistance to anthrax by Algerian sheep, where as sheep in general are susceptible to anthrax. It has been reported that the Negroes in the USA are more susceptible to tuberculosis than the whites. An interesting instance of genetic resistance to *Plasmodium falciparum* malaria is seen in some parts of Africa, where sickle cell anemia is prevalent. The hereditary abnormality of the red cells confers immunity to infection by malaria parasite. Even resistance to human diseases, such as measles, can vary from person to person. For example, although the effect of measles is usually relatively mild in European ancestry, the disease devastated the population of Pacific Islanders, when they were first exposed to measles by European explorers. Natural selection resulting from the exposure of many generations to the measles virus, presumably led to the more frequent inheritance of genes that conferred some resistance to the virus.

Individuals in a race exhibit difference in innate immunity. The genetic basis of individual immunity is evident from studies on the incidence of infectious disease in twins. Homozygous twins exhibit similar degree of resistance or susceptibility to lepromatous leprosy and tuberculosis. An individual's resistance to disease also depends on age, nutritional status, stress, hormone influence and general health in addition to genetic factor.

*Age:* Two extremes of life carry higher susceptibility to infections in comparison to adults. The heightened susceptibility of the fetus to infection is related to the immaturity of the immune system. In neonates, the antibodies, immune competent cells and also the complement level remain suboptimal. The fetus in uterus is normally protected by the maternal antibody, but some organisms (*Toxoplasma gondii*, rubella virus, cytomegalovirus, herpesviruses, *Treponema pallidum, Borrelia burgdorferi,* hepatitis B virus, human immunodeficiency virus, etc.) cross the placental barrier and cause respective diseases. Newborn animals (suckling mice) are more susceptible to coxsackievirus.

Tinea capitis caused by *Microsporum audouinii* is very common in young people, which disappear after reaching puberty. The vaginal epithelium of prepubertal girl is more susceptible to gonococcal infection.

Some infections like poliomyelitis and chickenpox, tend to be more severe in adults than in young children due to hypersensitivity that causes more tissue damage. The old people are prone to infection due to waning of the immune system. The immune system shows the senescence seen in other organs. Cellular immunity is most affected.

*Hormonal influence:* Diabetes, hypothyroidism and adrenal dysfunctions are associated with enhanced susceptibility to infections. Corticosteroids depress host resistance by anti-inflammatory and antiphagocytic effects and also by suppressing antibody formation. The elevated steroid level in pregnancy may have a relation to the heightened level of susceptibility to the staphylococcal infection.

*Nutrition:* The interaction between malnutrition and immunity is very complex. But in general, malnutrition depresses both cell-mediated immunity (CMI) and antibody-mediated immunity (AMI). CMI and AMI responses to T cell-dependent antigens are primarily reduced in malnutrition.

Paradoxically, there are some evidences that the infections may not be clinically apparent in ill nourished and malnourished patient. Fever in malaria may not be induced in famine-stricken area, but once that nutrition is improved fever appears. Some viruses may not multiply in the tissue of several malnourished individual.

*Stress:* Whether psychological or physical, stress adversely affects the immune response.

### *Mechanism of Innate Immunity*

### First line of defense

*Physical barriers:* Skin and mucous membrane form an important line of defense. Intact skin is impenetrable to most of the bacteria. Its low pH and presence of fatty acid makes the environment inhospitable for bacteria other than commensals. The continual shedding of the squamous epithelium also reduces bacterial load. If the continuity of the skin is compromised, the skin may be secondarily infected.

The mucous membranes form a less formidable barrier. The mucus with entrapped bacteria is swept away by cilia of the ciliated respiratory mucosa or the villi in the intestine particles are swallowed and coughed out by cough reflex.

The flushing effect of the body secretions reduces the microbial flora. Any slowing of urinary flow increases the chance of ascending infection. Saliva teeming with oral bacteria flows to the back of throat and is swallowed; gastric acidity destroys most swallowed bacteria. Commensal flora in the intestine prevents the colonization by pathogenic bacteria.

*Chemical factors (Antimicrobial substances):* The barrier defense of skin and mucous membrane are reinforced by the presence of antibacterial substances.

Lysozyme, a hydrolytic enzyme, found in the mucus secretions and in tears, is able to cleave the peptidoglycan of the bacterial cell wall. Saliva contains antibacterial hydrogen peroxide ($H_2O_2$). The low pH of stomach and vagina is inimical to most bacteria. Cholera infection occurs more rapidly in association with achlorhydria.

Several substances, possessing antimicrobial property, have been described in blood and tissue. These include:

1. Beta-lysine active against anthrax and related bacilli.
2. Basic polypeptides (leukin from leukocytes and plakin from platelets).
3. Lactic acid found in the muscle tissue and in the inflammatory zone.
4. Lactoperoxidase in the milk.
5. Virus inhibiting substances (antiviral substances) inhibit viral hemagglutinin.
6. A cysteine-rich peptide called defensins secreted by a variety of cells (epithelial cells, neutrophils, macrophages) in the skin and mucous membrane.
7. Other molecules with microbicidal functions include cathelicidin, deoxyribonuclease (DNases) and ribonuclease (RNases).
8. Acute phase proteins (Table 3.1).

In an acute phase of infection, pathogens ingested by macrophages stimulate the synthesis and secretion of several cytokines. Cytokines such as interleukin-1 (IL-1) and IL-6 travel through the blood and cause the liver to synthesize and secrete acute phase proteins into the blood.

*Interferons:* A method of defence virus infection is the production of interferon (IFN) by cells stimulated by live or killed viruses and certain other inducers. IFN has been shown to be more important than specific antibodies in protection against and recovery from certain acute viral infections.

*Immunoglobulin:* All classes of immunoglobulins (Ig) have been detected on mucous membranes, but IgA is the most important, because it is present in the greatest amount. IgA is a dimer, linked by secretory piece that not only aids transport, but also renders it resistant to proteolytic enzymes in the secretions. IgA is not involved in complement -mediated killing (classical pathway), but impedes adherence, an essential first step in colonization.

*Complement system:* The complement is a group of serum proteins that circulate in an inactive state. A variety of specific and non-specific immunologic mechanisms can convert the inactive form of complement proteins into an active form leading to lysis of bacteria, cells and viruses; promotion of phagocytosis (opsonization); triggering of inflammation; secretion of immune-regulatory molecules and clearance of immune complex from the circulation. In the innate immune system, complement can be activated by alternative pathway or via the mannan-binding lectin (MBL) pathway.

**Table 3.1** Role of acute phase proteins in innate immunity

| Acute phase proteins | Role in resistance |
|---|---|
| C-reactive protein | Stimulate and modulate inflammations |
| α1-acid glycoprotein | Improved wound healing |
| α1-trypsin | Control tissue damage by leukocyte proteins |
| α1-macroglobulin | Increased granulopoiesis and macrophage activation |
| Complement | Promotes phagocytosis |
| Haptoglobin | Ingestion of complexes by macrophages, remove iron source from bacteria |
| Fibrinogen | Coagulation |
| Lipopolysaccharide-binding protein (LPS-BP) complex | More powerful activator of macrophages |

*Cytokines and chemokines:* The cytokines are secreted by leukocytes and other cells and are involved in innate immunity, adaptive immunity and inflammation. Cytokines act in an antigen non-specific manner, triggering a wide range of biological activities from chemotaxis to activation of specific cells. Chemokines are subgroups of cytokines of low molecular weight involved in chemotaxis (chemical-induced migration).

*Commensal flora:* It prevents colonization by pathogens. Alteration of normal resident flora may lead to invasion by extraneous microbes causing serious disease such as staphylococcal and clostridial enterocolitis following antibiotics. Commensals protect the host by various mechanisms:

1. Competition for available food and tissue receptors.
2. Production of toxic substances, such as fatty acids or antagonistic substance such as bacteriocins.
3. Stimulation of antibodies (natural antibody that may cross react with pathogens).
4. Keeping the immune system primed, so that the monocytes bear class II histocompatibility antigens needed for immune response.

### Second line of defense

When the first line of defense fails, either because of congenital or acquired defects, then the way to deeper tissue is open to bacteria and the next lines of defense come to play. Ciliary dysfunction associated with respiratory infections is one of the congenital defects. There are many examples of acquired defects, the increasing use of indwelling devices provides niches for bacterial colonization and infection. Bacteria (e.g. *Staphylococcus epidermidis*) grow on these foreign bodies in a biofilm, protect them from host defense.

*Cellular factors in innate immunity:* Natural defense against the invasion of the blood and tissue is mediated by phagocytic cells. Phagocytosis is the process by which the invading organisms are ingested by phagocytic cells, ingestion being followed by intracellular killing. Many cells are able to ingest the

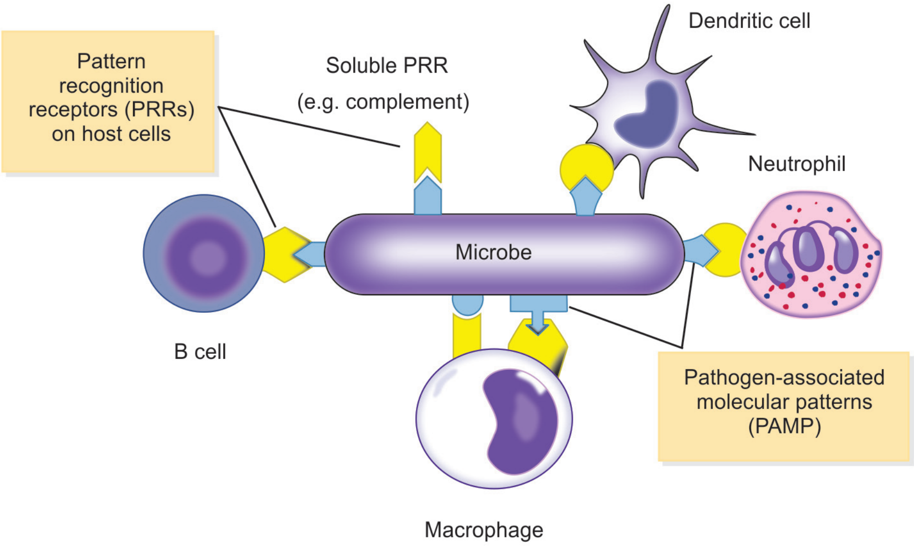

Pattern recognition receptors (PRRs) on host cells
Soluble PRR
(e.g. complement)
Dendritic cell
Neutrophil
Microbe
B cell
Pathogen-associated molecular patterns (PAMP)
Macrophage

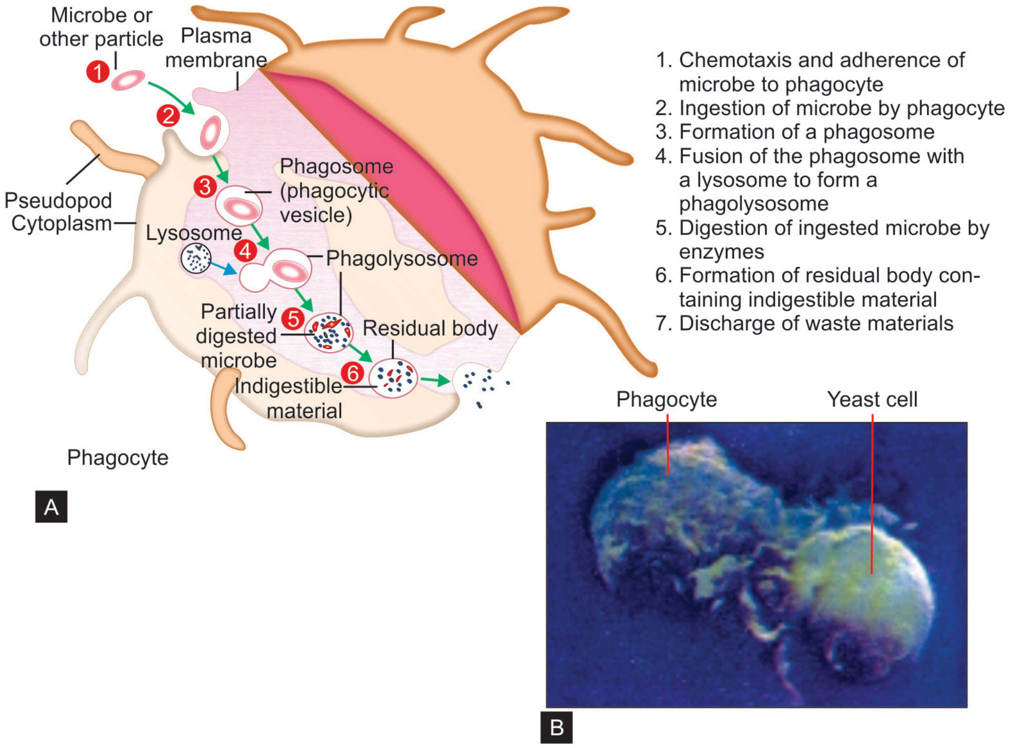
**Fig. 3.2:** Pattern recognition receptors (PRRs). PRRs detect and bind pathogen-associated molecular patterns (PAMPs).

particles, e.g. endothelial cells, but three cells may be regarded as professional phagocytes. These are neutrophils, macrophages and to a much lesser degree eosinophils. Macrophages consist of histiocytes (wandering ameboid cells found in the tissue), the fixed reticulo -endothelial cells and blood monocytes.

The innate immune system provides a rapid, initial means of defense against infection using genetically programmed receptors that recognize these structural features of microbes that are not found in the host. Such receptors are known as pattern recognition receptors (PRRs), which are found on or in phagocytic cells, which bind to pathogen-associated molecular patterns (PAMPs) (Fig. 3.2). PAMPs are conserved, microbes-specific carbohydrates, proteins, lipids and/or nucleic acid (e.g. lipopolysaccharide, peptidoglycan, etc.). PRRs binding to PAMPs result in phagocytosis and enzymatic degradation of the infectious organisms (Figs 3.3A and B).

Pattern recognition receptors engagement can lead to activation of the host cell and its secretion of antimicrobial substances. PRRs include:

1. Toll-like receptors (TLRs), which signals the synthesis and secretion of cytokines to promote inflammation by recruiting cells.
2. Scavenger receptors that are involved in internalization of bacteria and phagocytosis of host cells that are undergoing apoptosis.
3. Opsonins, the molecules (C3a, IgM), which bind to microbes to facilitate their phagocytosis.


Microbe or other particle
Plasma membrane
1
2
Pseudopod
Cytoplasm
3
Phagosome (phagocytic vesicle)
Lysosome
4
Phagolysosome
Partially digested microbe
5
Residual body
6
Indigestible material
Phagocyte
A
1. Chemotaxis and adherence of microbe to phagocyte
2. Ingestion of microbe by phagocyte
3. Formation of a phagosome
4. Fusion of the phagosome with a lysosome to form a phagolysosome
5. Digestion of ingested microbe by enzymes
6. Formation of residual body containing indigestible material
7. Discharge of waste materials
Phagocyte
Yeast cell
B

**Figs 3.3A and B:** Mechanism of phagocytosis in a phagocyte. **A.** Phases of phagocytosis; **B.** Phagocyte engulfing an yeast cell.

The cells under stress, either by infection or by cancerous change, express certain stress molecules (heat shock protein, MICA and MICB on the surface of the cells). These stress signals are detected by various receptors, including some of the TLRs (e.g. TLR2, TLR4) and the killer activation receptors (KARs) on the natural killer (NK) cells. Killer inhibition receptors (KIRs) on NK cells assess major histocompatibility complex class I (MHC I) molecules on the target cell surface. NK cells bring about the death of organisms (viruses) and tumor cells not by intracellular digestion, but by extracellular killing by liberating perforin, a cytolysin after degranulation (Fig. 3.4). The activity of NK cells is greatly increased by exposure to IFNs and cytokines.

*Inflammation:* Tissue injury, initiated by the entry of pathogens leads to inflammation, which is an important non-specific mechanism of defense. Hence, inflammation acts as a protective phenomenon.

1. Blood flow to the particular part is increased.
2. There is an outpouring of plasma, which dilutes the toxins and enzymes.
3. Chemotactic factors including C5a, histamine, leukotrienes, etc. will attract phagocytic cells to the site. The increased vascular permeability will allow easier access for neutrophils and monocytes. Vasodilation means more cells in the vicinity.

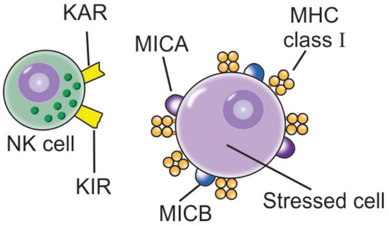


**Fig. 3.4**: Killer cell activation receptors (KARs) and killer cell inhibition receptors (KIRs) are expressed on NK cells. On nucleated cells, KARs detect stress-related molecule, MICA and MICB, while KIRs detect MHC class I molecules.

4. There is formation of fibrin barrier, which limits the inflammation.
5. There is activation of complement and also the specific defenses.

*Fever:* A rise of temperature following infection, helps in following ways:

1. Mobilization defenses.
2. Accelerate repairs.
3. Inhibits pathogens.
4. Stimulates the production of IFNs and helps in recovery from virus infection.

Therapeutic induction of fever was employed previously for destruction of *T. pallidum.*

The mechanisms of all the innate immunity is given in Figure 3.5.

## Acquired Immunity

The resistance an individual acquires during life is called acquired immunity.

### *Active Immunity (Adaptive Immunity)*

Active immunity or adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules, i.e. tumor antigens, transplanted antigens, etc. This involves the active functioning of the individual's immune apparatus, either in producing antibody or creating immune-competent cells for cell-mediated immunity (CMI). Active immunity sets in only after a latent period, which the immunological machinery needs for its functioning. Once developed the active immunity is long lasting. When the individual is facing the same antigen subsequently, there is no latent or lag phase and the immune response is prompt, powerful and prolonged (Table 3.2).

In contrast to the innate immune response, which recognize the common molecular patterns such as PAMPs in potential invaders, the adaptive immune system resorts to a highly different approach with a very large repertoire of specific antigen receptors that

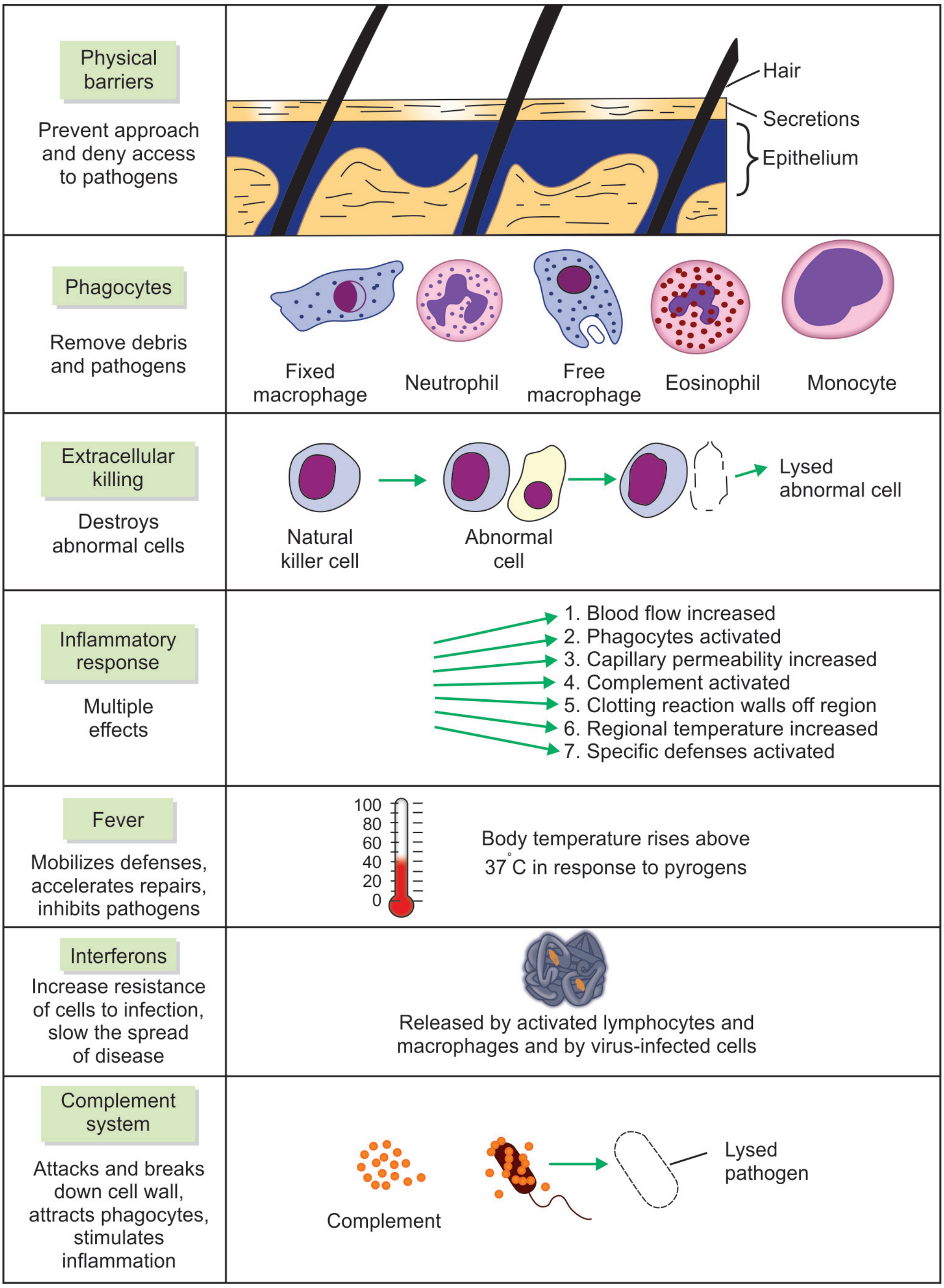

Physical barriers
Prevent approach and deny access to pathogens
Hair
Secretions
Epithelium
Phagocytes
Remove debris and pathogens
Fixed macrophage
Neutrophil
Free macrophage
Eosinophil
Monocyte
Extracellular killing
Destroys abnormal cells
Natural killer cell
Abnormal cell
Lysed abnormal cell
Inflammatory response
Multiple effects
1. Blood flow increased
2. Phagocytes activated
3. Capillary permeability increased
4. Complement activated
5. Clotting reaction walls off region
6. Regional temperature increased
7. Specific defenses activated
Fever
Mobilizes defenses, accelerates repairs, inhibits pathogens
100
80
60
40
20
0
Body temperature rises above 37°C in response to pyrogens
Interferons
Increase resistance of cells to infection, slow the spread of disease
Released by activated lymphocytes and macrophages and by virus-infected cells
Complement system
Attacks and breaks down cell wall, attracts phagocytes, stimulates inflammation
Complement
Lysed pathogen

**Fig. 3.5:** Mechanism of innate immunity

can recognize virtually, any component of the foreign invader.

Adaptive immunity focuses on four important characteristic features. They are:

1. Antigenic specificity.
2. Diversity.
3. Immunological memory.
4. Self/non-self recognition.

The antigenic specificity of the immune system permits it to distinguish minor difference among antigens. The antibodies can distinguish between two protein molecules that differ in only a single amino acid. The immune system is capable of generating tremendous diversity in its recognition molecules, permitting to recognize vast arrays of unique structures on foreign antigens. Once the immune system recognized and responded to an antigen, it exhibits immunological memory to recognize the same antigen, subsequently and react in a heightened manner (Refer Table 3.2). Finally, the immune system, normally responds to foreign antigens, indicating that it is capable of distinguishing self from non-self.

The adaptive immunity is not independent of innate immunity. They interact constantly. The phagocytic cells crucial to nonspecific immune responses are intimately involved in igniting the specific immune response. Conversely various soluble factors produced during specific immune response have been shown to augment the activity of these phagocytic cells.

*Naturally acquired active immunity:* This type of immunity is obtained when a person is exposed to antigens in the course of daily life. Once acquired, the immunity lasts for rest of its life such as in measles and chickenpox. For other diseases, especially in intestinal diseases, the immunity is short lasting. Subclinical infections can also conform immunity as that occurs in tuberculosis. Adults have natural immunity against polio after repeated subclinical infections.

In syphilis, malaria and few other diseases, a special type of immunity is observed known as infection immunity (premunition). The immunity to reinfection lasts as long as the original infection persists.

**Table 3.2** Comparison of active and passive immunity

| | Active | Passive |
|---|---|---|
| Mechanism | Produced actively by the host immune system | Obtained passively, no participation |
| Induction | Induced by infection (clinical and subclinical)<br>Induced by immunogens, vaccines | Conferred by ready-made antibody |
| Durability | Protection is durable and effective | Protection is transient and less effective |
| Lag phase | Present | No lag phase |
| Immunological memory | Present, subsequent challenge is more effective | No immunological memory, hence no secondary response |
| Negative phase | May occur | No negative phase |
| Application to immune deficient subjects | Not applicable | Effective in immune deficient hosts |

*Artificially acquired active immunity:* This type of immunity results from vaccination or immunization. Vaccinations may be inactivated bacterial toxins (toxoids), killed microorganisms, live but attenuated microorganisms or parts of microorganisms such as capsules.

These substances can no longer cause disease, but can stimulate immune response.

Examples of vaccines are as follows:

1. Bacterial vaccines

Live and attenuated → Bacille Calmette-Guérin (BCG) for tuberculosis

Killed → Typhoid-paratyphoid A and B vaccine (TAB) for enteric fever
Taboral vaccine for typhoid (oral vaccines)
Cholera vaccine
Pertussis vaccine
Bacterial capsule polysaccharides

- *Haemophilus influenzae*
- *Pneumococcus*
- *Meningococcus.*

Subunit → Vi polysaccharide for typhoid (Vi virulence)

Bacterial products → Toxoids for diphtheria tetanus

Bacterial products and killed bacteria → Triple vaccine.

2. Viral vaccines

Live and attenuated → Smallpox
Oral polio (Sabin)
Influenza
Measles, mumps, rubella (MMR)

Killed (inactivated) → Injectable
Polio (Salk)
Yellow fever
Influenza
Rabies

Subunit → Hepatitis B vaccine.

Live vaccines initiate an infection without causing any injury or diseases. The immunity lasts for several years. Booster dose may or may not be required. Live oral (polio) or nasal spray (influenza) vaccines provide local immunity.

Killed vaccines are generally less immunogenic than the live vaccines and the immunity lasts only for a short period. Therefore, they are administered repeatedly. Killed vaccines are given parenterally.

### *Passive Immunity*

Passive immunity is resistance exhibited by the host, when ready-made antibodies or defensive cells are introduced into the body. This form of protection is passive, because the individuals own immune system does not make antibodies or defensive cells against the disease producing agents or toxins.

*Naturally acquired passive immunity:* This type of immunity involves natural transfer of antibodies from mother to her infant and also from mother to fetus. Certain antibodies (IgA) are passed from the mother to her nursing infants in breast milk, especially in the first secretion called colostrum. The immunity in infants last as long as baby feeds on breast milk.

During pregnancy, some of the maternal antibodies are also transferred through placenta to the fetus. If the mother is immune to diphtheria, rubella or polio, the newborn will be temporarily immune to these diseases as well.

*Artificially acquired passive immunity:* This type of immunity involves the introduction of antibodies into the body. These antibodies come from animal or person, who is already immune to the disease. They are:

1. Hyperimmune sera of animal or human origin. Common examples are antitetanus serum (ATS), antidiphtheria serum (ADS), anti-gas-gangrene serum (AGS), antisnake venom, etc.
2. Convalescent sera from patients very recently recovered from measles, rubella, etc.
3. Pooled gamma globulin serum against common infectious diseases.
4. Human gamma globulin is also used in the treatment of immunodeficiency diseases.

#### *Indication of Passive Immunization*

1. For providing immediate and temporary protection in a non-immune host.
2. Treatment of some infections.
3. Antilymphocytic serum (ALS) may be given for suppression of lymphocytes in transplantation surgery.
4. Passive immunization may also be employed to suppress active immunity, when the latter may be injurious. The commonest example is the use of Rh immunoglobulin during delivery to prevent immune response to rhesus factor in Rh-negative women with Rh-positive babies.
5. Combined immunization.

At times, both active and passive immunization is given together. Ideally, it is employed to provide immediate protection to non-immune individual with a tetanus-prone wound. Tetanus immunoglobulin (TIG) will provide immediate passive immunity and toxoid will initiate active immunity.

## ADOPTIVE IMMUNITY

Adoptive immunity is a special type of immunization, where the immunocompetent cells are injected. At times, instead of whole lymphocytes, an extract of lymphocytes (transfer factor of Lawrence) may be introduced as a therapeutic procedure in certain disease, such as lepromatous leprosy, immunodeficiency diseases such as Wiskott-Aldrich syndrome, disseminated malignancy, etc. Recent studies indicate that the adoptive T cell transfer is done, preferably, in the treatment of virus-related diseases in particular, cytomegalovirus (CMV) infection and Epstein-Barr virus (EBV) infection—associated lymphoproliferative diseases (LPDs).

### Local Immunity

The mucosal immune system is composed of the lymphoid tissues that are associated with the mucosal surface of the gastrointestinal, respiratory and the urogenital tracts. These include mucosal-related Igs. The system involves production of mucosal-related Ig that is IgA (secretory immunoglobulin). The primary function of the mucosal immune system is to provide defense to the host at mucosal surface, locally. Optimal host defense at the mucosal surface depends on both intact mucosal immue responses and non-immunologic protective functions such as residential bacterial flora, mucosal motor activity (peristalsis; ciliary function), mucus secretion that create barrier between potential pathogens and epithelial surfaces and innate immunity factors (lactoferin, lactoperoxidase and lysozyme).

The concept local immunity has gained importance in the treatment of infections, which are either localized or where it is operative in combating infection at the site of primary entry of pathogens.

The Sabin vaccine (for poliomyelitis) is administered orally to promote local IgA (secretory IgA) production in the intestinal tract. This prevents entry and multiplication of the organism.

Influenza (live-attenuated) vaccine is most effective when given in nasal spray. Research to find out an oral vaccine against cholera is on the process.

## Herd Immunity

Herd immunity refers to an overall immunity exhibited by a community, which is relevant in the control of epidemic diseases. When the herd immunity is satisfactory, epidemic does not occur. When it is less, the prevention of epidemic can be done by mass immunization.

There are limits to the herd immunity. However, if a significant number of unprotected individuals become infected, the infection could spread rapidly through the unprotected members of population. In the course of that rapid replication, new mutant forms might arise that could evade the immune response and produce diseases in vaccinated individuals as well.

## STUDY QUESTIONS

### Essay Questions

1. Define innate immunity. Explain various innate immune mechanisms.
2. Classify immunity. Describe acquired immunity with examples.

### Short Notes

1. Artificially acquired active immunity.
2. Killed vaccines.
3. Live-attenuated vaccines.
4. Oral vaccines.
5. Herd immunity.
6. Premunition.
7. Pattern recognition receptors (PRRs).

## SUGGESTED READING

1. Black JG. Microbiology: Principles and Applications, 3rd edition; 1996.
2. Goldsby RA, Kuby TJ. Immunology, 4th edition; 2000.
3. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
4. Janeway CA, Travers P. Immunobiology. London: Current Biology; 1994.
5. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. Lange Basic Science. USA: McGraw Hill; 2010.
6. Peakman M, Vergani D. Basic and Clinical Immunology, 1st edition; 1997.
7. Schaechter M, Cary N, Barr I. Mechanisms of Microbial Disease, 3rd edition; 1998.
8. Stites. Basic and Clinical Immunology, 8th edition; 2007.
9. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's Illustrated Reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
10. Tortora, Funke, Case. Microbiology, 8th edition. Edinburgh: Churchill Livingstone; 1997.

# Antigen

# 4

## DEFINITION

The antigen is any substance capable of provoking the immune system of an animal or a person to respond by generating an immune reaction, specifically directed at the inducing substances and not at other unrelated substances.

The response may involve exclusively the humoral or cellular limb of the immune system, but most commonly involves both. As a rule, the immune response is carried out by only those B cell and T cell clones, whose surface immunoglobulin (Ig) or T cell receptor (TCR) protein can serve as the target of immune response.

The recognition of antigen by T cells and B cells is fundamentally different. B cells recognize soluble antigen when it binds to their membrane bound antibody. But T cells recognize peptides (antigen) combined with major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APC). Therefore, the T cells epitope includes the combination of peptides and MHC molecules. In the context of this explanation, the antigen can be defined as the substance that can be recognized by the Ig, receptor of B cells or by TCR complex with MHC molecule.

## EPITOPES

Antigen receptors recognize discrete regions of molecules called antigenic determinants or epitopes, the smallest part of an antigen that is seen by B cell receptors (BCR) and TCR. Different lymphocytes, each with different set of receptors, recognize different epitopes on the same antigen. BCR can recognize their specific epitopes, whether they are a part of free soluble molecules, surface-bound molecules or even degraded fragments of antigen. The TCR can bind to epitopes, consisting of peptide-MHC molecule combinations, presented on the surface of an APC.

Based upon the nature of immune responses they generate, the antigens/epitopes are divided into three broad functional categories:

1. Immunogens.
2. Haptens.
3. Tolerogens.

### Immunogens (Complete Antigens)

Immunogens are antigens/epitopes that induce immune response either by producing antibody or sensitized lymphocytes, which in turn react specifically with immunogens, which produced them. Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules called haptens are antigenic, but incapable by themselves of inducing specific immune response.

### Haptens (Partial Antigens)

Haptens are small molecular weight substances, which are antigenic, but incapable by themselves of inducing specific immune response. In other words, they lack immunogenicity, but can react with specific antibody. Penicillin is a good example of hapten. The drug is not immunogenic by itself, but some people develop hypersensitivity reaction to it. In these people, when penicillin combines with serum protein, the resulting combined molecules initiate an immune reaction (Fig. 4.1).

### Tolerogens

Tolerogens are antigens (usually self), which induce in normal condition, immune unresponsiveness. During development of immune repertoire, tolerance to self-molecules and cells develop first. Therefore, there is no immune response against the self-tissue in normal healthy state.

## CLASSIFICATION OF ANTIGENS

### Based on Origin

Based on origin, antigens are classified into following types.

- Microbial
- Fimbrial
- Somatic
- Flagellar
- Capsular.

### Based on Immune Response

*T-independent Antigen*

- Stimulate specific immunoglobulin production without the help of T lymphocytes, e.g. large molecular weight antigens with regularly repeating epitopes (pneumococcal polysaccharide, bacterial lipopolysaccharide, fimbrial and flagellar antigen) (Fig. 4.2)
- IgM is the main antibody
- Short memory.

*T-dependent Antigens*

- Do not stimulate antibody production without the help of T lymphocytes, e.g. serum proteins, erythrocytes, haptens, etc.
- IgM, IgG, IgA and IgE are the antibodies
- Long memory.

## DETERMINANTS OF IMMUNOGENICITY

Antigens vary widely in degree to which they are immunogenic. Immunogenicity is determined primarily by following factors.

### Molecular Weight

The most potent immunogens are usually large proteins. Large molecules (hemocyanins, molecular weight 6.75 million) are highly antigenic. Molecular weight less than 10,000 are weakly immunogenic. They become immunogenic only when linked to carrier protein.

### Chemical Complexity

Protein and polysaccharide are more antigenic. Lipid and nucleic acid are less immunogenic than heteropolymer containing three or more different amino acids. Not all proteins are however antigenic. A well known exception is gelatin. Presence of aromatic radical is essential for antigenicity. In gelatin, there are no aromatic amino acids such as tyrosine.

T cells recognize peptide-derived protein antigens when they are presented as peptide-MHC complex. Lipoidal compounds such as glycolipids and some phospholipids can be recognized by TCR when presented as complex with molecules that are almost similar to MHC molecules structurally. These lipid-presenting molecules are members of cluster

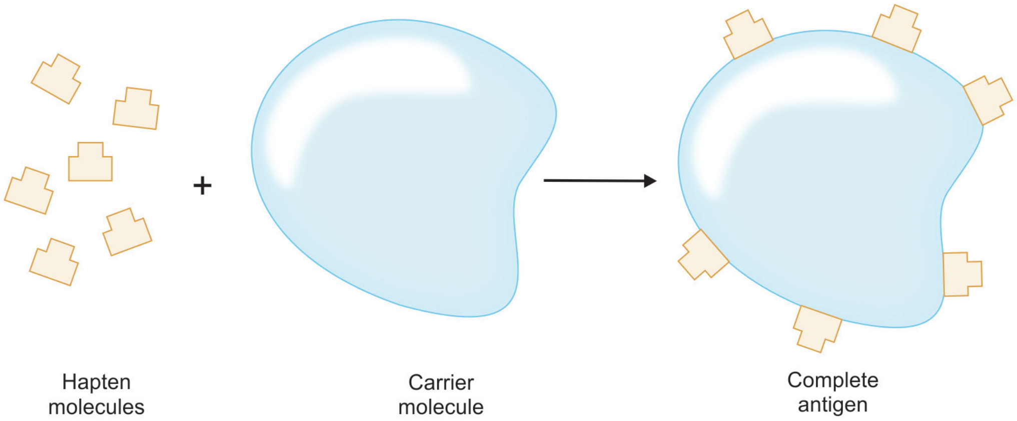


**Fig. 4.1:** Haptens: A hapten is a molecule too small to stimulate antibody formation by itself. However, when the hapten is combined with a larger carrier molecule, usually a serum protein, the hapten and its carrier together function as an antigen and can stimulate an immune response.

of differentiation (CD1) family. Recognition of lipid by T cells, as a part of immune response to some pathogens (*Mycobacterium tuberculosis, M. leprae*) has been documented.

### Foreignness (Difference from Self)

An important function of the immune system is to distinguish self (host) from non-self (foreign). Therefore, the more dissimilar a molecule from host molecules, the greater its immunogenicity.

### Antigenic Determinants (Epitopes)

An antigen may have one or more antigenic determinants (a determinant is roughly 5 amino acids or sugars in size). More the number of epitopes are there, they are antigenic not immunogenic (fails to activate T lymphocytes and B lymphocytes).

### Genetic Constitution of the Host

Two members of the same species of animals may respond differently to the same antigen, because of a different composition of immune response genes.

### Susceptibility to Tissue Enzymes

Substances, which are metabolized and are susceptible to enzymic action, are antigenic or immunogenic. Polystyrene latex particles, synthetic polypeptides, etc. which are not metabolized are not antigenic.

### Dosage, Route and Timing of Antigen

Administration also determine the immunogenicity. It is possible to enhance the immunogenicity of a substance by mixing it with an adjuvant. Adjuvants are substances that maintain the continuous stimulation of the immune responsive cells by slow release.

## ANTIGEN RECEPTORS

The receptors, which recognize antigen and allow the antigen to bind are different in innate adaptive immune system. Antibodies BCR and TCR are somatically generated receptors, which perform important role in adaptive immune system. On the other hand the innate immune system uses preformed receptors, which are mostly found on the cell surface. These receptors recognize broad structural motifs that are highly conserved within the microbial species, but are generally absent in host. Because they recognize particular overall molecular patterns, such receptors are called pattern recognition receptors (PRRs). Many PRRs are present in bloodstream and tissue fluid as soluble circulating proteins. These include mannose-binding lectin (MBL), 'C' reactive proteins (CRPs), complement and lipopolysaccharide-binding proteins (LPBPs). PRRs detect and bind pathogen-associated molecular pattern (PAMP). PRRs found on the cell membrane of macrophages, neutrophils and dendritic cells include scavengers receptors (SRs) and toll-like receptors (TLRs). The SRs are involved in the binding and internalization of gram-positive bacteria and gram-negative bacteria and phagocytosis of apoptotic cells. The TLRs (TLR1–TLR11) mediate cytokine production to promote inflammation and trafficking of immune cells to the site (Refer Fig. 3.2). The other preformed receptors, besides PRRs, which take part in innate immunity include killer activation receptor (KAR), killer inhibition receptor (KIR), complement receptors, fragment crystallizable (Fc) receptors on phagocytic cells, etc. (Refer Fig. 3.4).

## ANTIGENIC SPECIFICITY

The basis of antigenic specificity is stereochemical, as was first demonstrated by Obermayer and Pick and confirmed by Landsteiner. Using hapten (atoxyl) coupled with protein, it was seen that antigenic specificity is determined by a single chemical grouping even by a single acid radical. The importance of position (ortho, meta and para) of the antigenic determinants in antigen molecules is also responsible for antigenic specificity. Antigenic specificity is not absolute. Cross reaction can occur between related species.

The specificity of natural tissue antigens of animals may be of various types:

1. Species specificity.
2. Isospecificity.
3. Autospecificity.
4. Organ specificity.
5. Heterogenetic specificity.

### Species Specificity

Tissues of all individuals, in a single species, share a common antigen. Cross reaction may occur in related species due to antigenic similarity. The species specificity is of immense help:

a. In tracing evolutionary relationship.
b. In forensic application in the identification of species of blood and seminal fluid.

### Isospecificity

Isoantigens are found in some, but not in all members of a species. The best examples of isoantigens are human erythrocyte antigens based on which different individuals are classified into different blood groups. These are

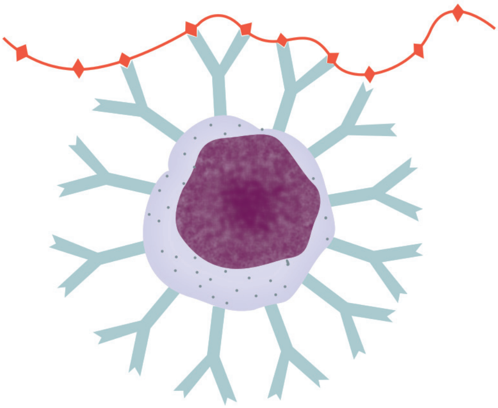


**Fig. 4.2:** T-independent antigens. T-independent antigens have repeating units that can cross-link several antigen receptors on the same B cell. These antigens stimulate the B cell to make antibodies without the aid of helper T cells. The polysaccharides of bacterial capsules are examples for this type of antigen.

genetically determined. These isoantigens, besides being of clinical importance in blood transfusion and isoimmunization in pregnancy, they are also helpful in providing valuable evidence in disputed paternity. Isospecificity also finds application in anthropology.

Histocompatibility antigens are those cellular determinants specific for each individual species. These are recognized by genetically different individual of the same species; when attempts are made to transfer or transplant cellular material from one individual to other. Histocompatibility antigens are associated with plasma membrane of tissue cells.

Several human and animal histocompatibility antigens have been identified. The major histocompatibility antigens determining the homograft rejection are the antigens of human leukocyte antigen (HLA) system. In mice, it is H2 complex system.

### Autospecificity

Autologous or self-antigens are ordinarily non-antigenic, but in certain circumstances self-antigens behave as foreign antigens. Lens protein has no access to circulation as confined inside the capsule (sequestrated antigens). Spermatozoa are absent in embryonic life, but subsequently develop in adolescent life. When these antigens are released into the circulation (by injury to lens or damage to the testis) antibodies are produced against them. Infection and irradiation may bring about change in antigenic specificity.

### Organ Specificity

Some organs such as brain, kidney, lens protein of different species share a common antigen. Such antigens are characteristics of organ- or tissue-specific antigens. The neuroparalytic complication following neural antirabies (sheep brain) vaccine is the result of cross reaction of organ-specific antigens.

### Heterogenetic Specificity

The same or closely related antigens may occur in different biological species, classes and kingdom. These antigens are known as heterogenetic or heterophile antigens. One of the examples of heterophile antigen is Forssman antigen. This is a lipid-carbohydrate complex widely distributed in human beings, animals, birds, plants and bacteria. Other heterophilic antigens, used in serological test, unrelated to causative agents are:

1. Proteus strains (OX-19, OX-2, OX-K) used as antigens in the diagnosis of typhus fever (Weil-Felix).
2. Sheep red cells used in the diagnosis of infectious mononucleosis caused by Epstein-Barr virus (Paul-Bunnell).
3. Red cell antigen in the diagnosis of primary atypical pneumonia caused by *Mycoplasma pneumoniae* (cold agglutination test).
4. Cardiolipin antigen for the diagnosis of syphilis caused by *Treponema pallidum* [standard test for syphilis (STS)].

## STUDY QUESTIONS

### Essay Questions

1. Define antigen, discuss about the determinants of antigenicity.
2. Write about the antigenic specificities.

### Short Notes

1. Hapten.
2. Epitope.
3. Heterophile antigen.
4. Organ specificity.
5. T-independent antigens.

## SUGGESTED READING

1. Black JG. Microbiology: Principles and Applications, 3rd edition. USA: Prentice Hall College div; 1996.
2. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
3. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
4. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
5. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. Lange Basic Science. USA: McGraw Hill; 2010.
6. Peakman M, Vergani D. Basic and Clinical Immunology, 1st edition. 1997.
7. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
8. Tortora, Funke, Case. Microbiology an Introduction, 6th edition. 1998.

# Antigen Recognition Molecules

# 5

In order for the immune system to respond to non-self, i.e. foreign antigen, a recognition system capable of precisely distinguishing self from non-self has to evolve. What are the molecules, which recognize and bind to antigen (foreign particles)?

1. Antibodies [immunoglobulins (Ig)], which include both:
   a. Membrane-bound receptor on the surface of B cells [B cell antigen receptor (BCR)], closely associated with secondary component such as Igα/Igβ heterodimer.
   b. Soluble molecules (secreted from plasma cells) present in serum and tissue fluids, which are structurally identical to the B cell antigen receptor, but lack transmembrane and intracytoplasmic portion.
2. T cell antigen-specific membrane-bound receptors (all the T cell population such as CD4, CD8, CD2, etc).
3. Products of major histocompatibility complex (MHC), includes both MHC I and MHC II. Class I MHC molecules bind to peptides, derived from cytosolic (intracellular protein), known as endogenous antigens. Class II MHC molecules bind to peptides derived from extracellular proteins that have been brought in to the cell by phagocytosis or endocytosis (exogenous antigens).

All the above stated molecules have structural features in common. They have a domain structure built on three dimensional features known as immunoglobulin fold (Ig fold). Their structure and functions so presumed to be members belonging to one gene family known as Ig supergene family (Fig. 5.1). Large numbers of other membrane proteins such as cell adhesion molecules [vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecules-2 (ICAM-2), leukocyte function antigen (LFA-3)], platelet-derived growth factor, β2-microglobulin, etc. are included in Ig supergene family.

## ANTIBODIES—IMMUNOGLOBULINS

The humoral basis of immunity was established at the early 18th century. Following injection of antigen into the animal, certain substances appeared in the serum and the tissue fluid called antibody, which reacted with the antigen specifically in an observable manner. Depending on the type of reaction, the antibodies were known as agglutinin, precipitin and complement-fixing antibodies and so on. Sera having a higher antibody level are known as immune sera.

Fractionation of immune sera by half-saturation with ammonium sulfate separated serum protein into soluble albumins and insoluble globulins. The insoluble globulin had

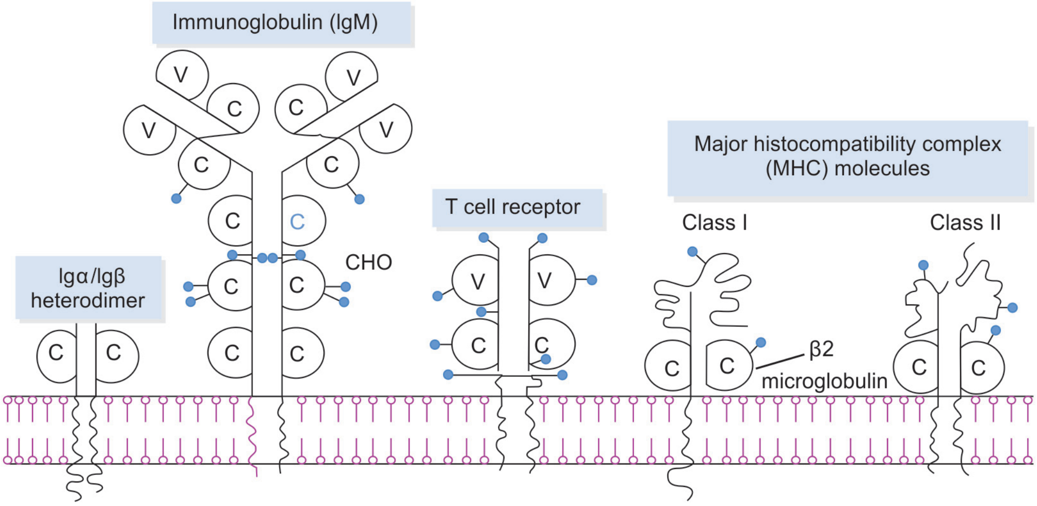


**Fig. 5.1:** Some members of the immunoglobulin supergene family, a group of structurally related usually membrane-bound glycoproteins

two fractions, water-soluble pseudoglobulin and another is the water insoluble, i.e. euglobulin. Most antibodies were found to be euglobulins.

Tiselius, in 1937 separated serum proteins by electrophoresis into albumin, alpha-globulin, beta-globulin and gamma-globulin. Antibody activity was associated with gamma-globulin.

Sedimentation studies using ultracentrifuge disclosed the diversity of the antibody molecules. While most were sedimented at 7S, molecular weight 150,000 (S = Svedberg unit, i.e. a sedimentation constant of $1 \times 10^{-13}$ second), some were heavier 19S (900,000).

Thus, indiscriminate use of various terminologies led to confusion. In 1964, a common terminology was evolved called Ig and was accepted internationally.

## Definition

Immunoglobulins are proteins of animal origin, endowed with known antibody activity and for certain other proteins related to them by chemical structure. That means the Ig include, besides antibody globulin, the abnormal proteins found in myeloma, macroglobulinemia, cryoglobulinemia, etc. While Ig satisfies the structural and chemical concept, the antibody provides biological and functional concept. All antibodies are Ig, but all Ig are not antibodies.

Immunoglobulins constitute 20% to 25% of the serum protein. Based on the physicochemical, antigenic differences and the types of heavy chain Igs are classified into five types.

All Igs are made up of light (molecular weight 25,000) and heavy polypeptide chains (molecular weight 50,000). Light (L) chains are of one of the two, kappa (K) or lambda (λ). Both types can occur in all classes of Ig (IgG, IgM, IgA, IgE and IgD), but any one Ig contains only one type of L chain. Both the L chain of one Ig molecule cannot have both kappa and lambda chain. The amino-terminal portion of each L chain contains a part of antigen-binding site.

Heavy (H) chains are distinct for each of the five Ig classes and are designated γ (gamma), μ (mu), α (alpha), δ (delta) and ε (epsilon). The amino-terminal portion of each H chain participates in the antigen-binding site. The carboxy-terminal portion forms the fraction crystallizable (Fc) fragment, which has various biologic activities (complement activation, macrophage fixation, reactivity with rheumatoid factor and binding to cell-surface receptors). An individual antibody molecule consists of two H chains and L chains, covalently linked by disulfide bonds. Both the H chains and L chains are identical.

Proteolytic cleavage of IgG by Porter, Edelman and their colleagues led to a better understanding of the detailed structure of the Ig molecule. Pepsin treatment produces a dimeric F(ab)$_2$ fragment. Papain treatment produces monovalent antigen-binding fragment (Fab) and Fc fragments. The F(ab)$_2$ and Fab fragments bind antigen, but lack a functional Fc region (Fig. 5.2).

Light and heavy chains are subdivided into variable regions and constant regions. A L chain consists of one variable domain ($V_L$) and one constant domain ($C_L$). Most H chains consist of one variable domain ($V_H$) and three or more constant domains ($C_H$). Each domain is approximately 110 amino acids long. Variable regions are for antigen-binding and the constant regions are responsible for other biologic functions.

In the variable regions of both L and H chains, there are three extremely variable (hypervariable) amino acid sequences that form the antigen-binding site. The hypervariable region (HVR) form the complementary region of the antigenic determinant and therefore, known as complementary determining regions (CDRs) (Fig. 5.3).

## Classes of Immunoglobulin

There are five classes of immunoglobulins, according to their properties (Table 5.1). They are:

1. Immunoglobulin G (IgG).
2. Immunoglobulin A (IgA).
3. Immunoglobulin M (IgM).
4. Immunoglobulin D (IgD).
5. Immunoglobulin E (IgE).

### *Immunoglobulin G*

Immunoglobulin G is the main class of immunoglobulin in serum. It exists as a

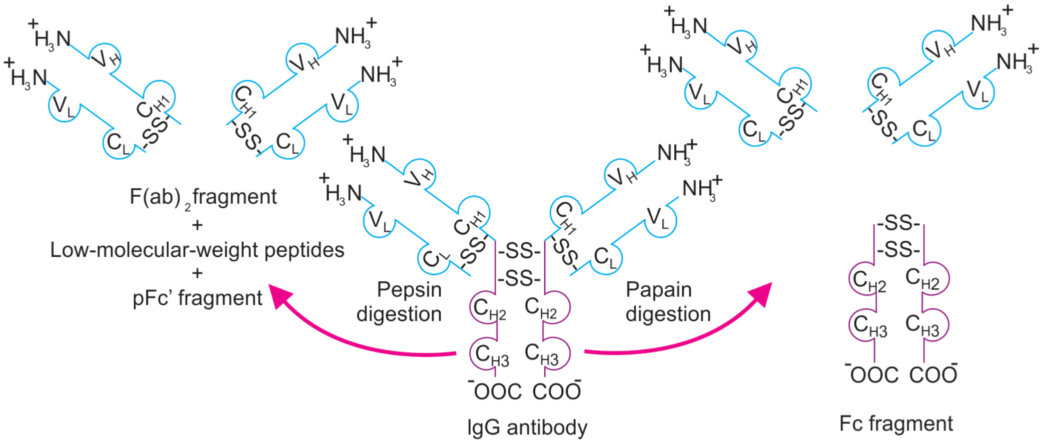


**Fig. 5.2**: Proteolytic digestion of immunoglobulin G (IgG). Pepsin treatment produces a dimeric F(ab)$_2$ fragment. Papain treatment produces monovalent Fab fragments and an Fc fragment. The F(ab)$_2$ and the Fab fragments bind antigen, but lack a functional Fc region.

**Table 5.1** Comparative features of immunoglobulin isotypes

| Property | IgG | IgA | IgM | IgD | IgE |
|---|---|---|---|---|---|
| Light chain | Kappa or lambda | Kappa or lambda | Kappa or lambda | Kappa or lambda | Kappa or lambda |
| Heavy chain | Gamma ($\gamma$) | Alpha ($\alpha$) | Mu ($\mu$) | Delta ($\delta$) | Epsilon ($\varepsilon$) |
| Serum concentration (mg/mL) | 12 | 2 | 1.2 | 0.03 | 0.0003 |
| Percentage | 75% | 10% –15% | 5% –10% | – | – |
| Sedimentation coefficient | 7S | 7S, 11S | 19S | 7S | 8S |
| Molecular weight | 150,000 | 160,000 | 900,000–1,000,000 | 180,000 | 190,000 |
| Half-life (day) | 23 | 6 | 5 | 3 | 2 |
| Placental transfer | + (IgG1, IgG3, IgG4) | – | – | – | – |
| Presence in milk | + | + | – | – | – |
| Carbohydrate percentage (%) | 3 | 8 | 12 | 13 | 12 |
| Heat stability (56°C) | + | – | + | + | – |
| Location (mostly) | Serum, extravascular | Transport across epithelium | Serum (intravascular) | B cell membrane | Serum, extravascular |
| **Principal biological activities** | | | | | |
| Complement fixation | + | + (alternate pathway) | +++ | – | – |
| Neutralization | + | – | – | – | – |
| Opsonization | + | – | +++ | – | – |
| Phagocytosis | + | + | –/+ | – | – |
| Binds to mast cells and basophils | – | – | – | – | + |
| Local immunity | – | + (secretory IgA) | – | – | – |
| Examples | Antiviral, antitoxic, opsonin, protects fetus and newborn | Immunity against enteric, polio and influenza virus | ABO red cell antibodies, rheumatoid factor, antibodies against OAg (Enterobacteriaceae) | – | Mediator of immediate-hypersensitivity reaction |

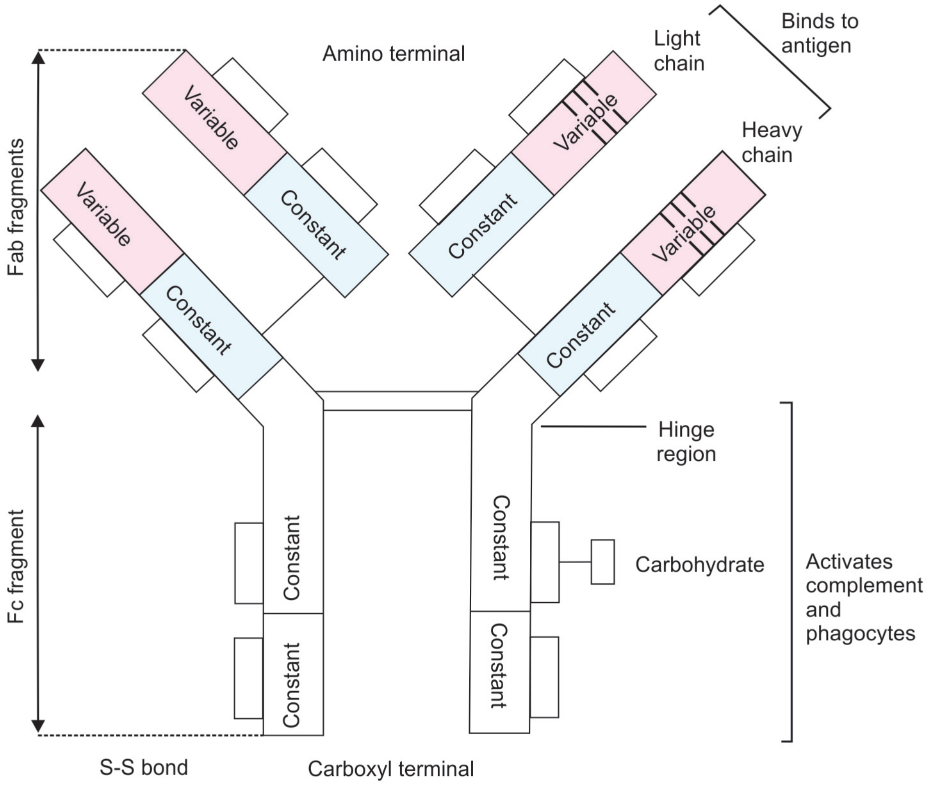

Amino terminal
Light chain
Binds to antigen
Heavy chain
Fab fragments
Variable
Variable
Variable
Variable
Constant
Constant
Constant
Constant
Hinge region
Constant
Constant
Constant
Constant
Carbohydrate
Activates complement and phagocytes
Fc fragment
S-S bond
Carboxyl terminal

**Fig. 5.3:** Schematic representation of an IgG molecule, indicating the location of the constant and the variable regions on the light and heavy chains

molecule of molecular weight 146 to 160 kDa (7S) in serum and is abundant component of the secondary humoral response. This class of Ig is not only found in the bloodstream, but also in extravascular spaces. It contains less carbohydrate than other Igs. It has a half-life of approximately 23 days. It is also transported across the placenta and is therefore, responsible for passive immunity in the fetus and neonate. Passively administered IgG, suppresses the homologous antibody synthesis by a feed back mechanism. This process is utilized in the immunization of women by the administration of anti-RhD IgG during delivery.

There are four subclasses of IgG isotypes in man (IgG1, IgG2, IgG3 and IgG4), each one is distinguished by a minor variation in the amino acid sequences in the C-region and by the numbers and location of disulfide bridges. The four subclasses are distributed in human serum, IgG1 (65%), IgG2 (23%), IgG3 (8%) and IgG4 (4%) (Fig. 5.4).

Immunoglobulin G participates in most immunological reactions such as complement fixation (IgG1 and IgG3), precipitation, neutralization of toxins and viruses. IgG1 and IgG3 are capable of interacting with the Fc receptors on macrophages and therefore, acting as efficient opsonins.

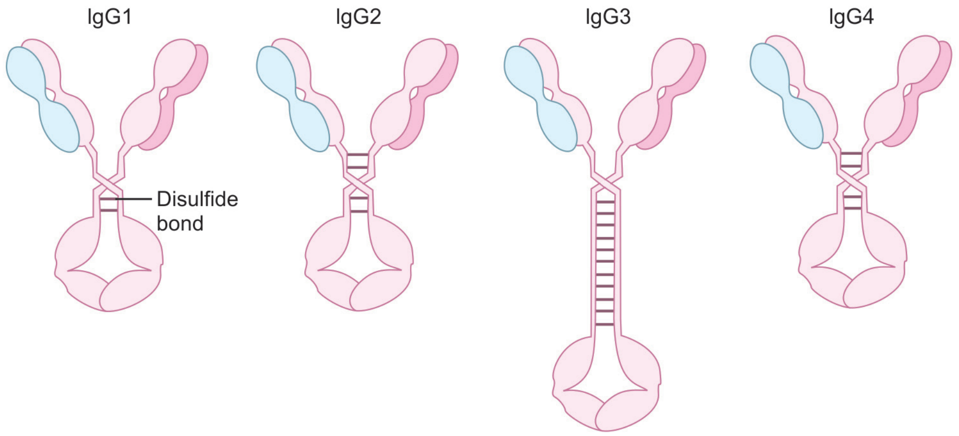


**Fig. 5.4**: General structure of the four subclasses of human IgG, which differ in the number and arrangement of the interchain disulfide bonds (thick brown lines) linking the heavy chains. A notable feature of human IgG3 is its 11 interchain disulfide bonds.

### *Immunoglobulin A*

Immunoglobulin A is the second most abundant class of immunoglobulin constitute about 10% to 13% of all serum immunoglobulins. The normal serum level is 0.6 to 4.2 mg per mL. It has a half-life of 6 to 8 days. IgA is found in two forms in the body—in serum, where it occurs principally as monomer (160 kDa, 7S) and on secretory surfaces, where it exists as a dimeric molecule (385 kDa, 11S).

The dimeric form is known as secretory IgA (sIgA) (Fig. 5.5) and is found in association of J chain and with secretory component; the latter is involved in the transport of IgA to the secretory surfaces. Secretory component is non-covalently associated with the IgA molecules in the sIgA complex. sIgA is the main Ig in the secretions such as milk, saliva and tears and in the secretions of respiratory, intestinal and genital tracts. It protects mucous membranes from attack by bacteria and viruses.

There are two subclasses of IgA; IgA1 and IgA2 distinguished by their distribution and arrangement of disulfide bonds. IgA is the predominant form found in serum, where as IgA1 and IgA2 isotypes are present in roughly equal amounts in IgA.

Immunoglobulin A is the component of the secondary humoral response. The principal antigens that elicit an IgA response are microorganisms in the gut or on the airways. IgA cannot cross the placental barrier, but however, sIgA can be passed to the neonate through milk. IgA does not fix complement, but can activate the alternative complement pathway. It promotes phagocytosis and intracellular killing of microorganisms.

### *Immunoglobulin M*

Immunoglobulin M constitutes 5% to 8% of serum Ig with a normal level of 0.5 to 2 mg per mL. It has a half-life of about 5 days. It is a heavy molecule (19S; molecular weight 900,000 to 1,000,000, hence called the millionaire molecule). It has a pentameric structure comprising five identical four chain units (Fig. 5.6), i.e. it has 10 identical binding sites. The ‘mu’ heavy chains has five domains, $V_H$ plus 4 C regions (Cμ1, Cμ2, Cμ3, Cμ4) and lacks a hinge region. The pentameric structure is stabilized by disulfide bonding

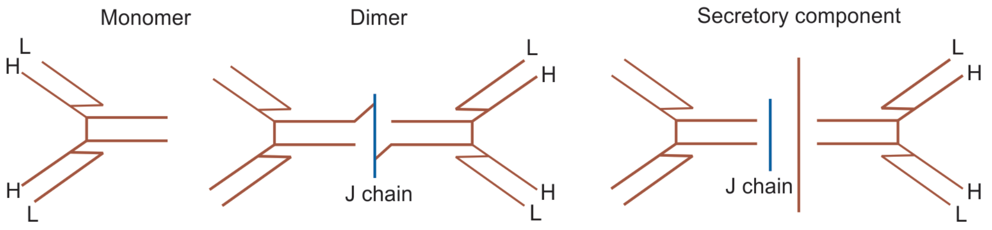


**Fig. 5.5**: Structure of IgA (both monomer and dimer)

between adjacent Cμ3 domains and by the presence of Joinez (J) chain. Though theoretically 10 antigen-binding sites are there, only five antigen-binding sites react with antigen probably due to steric hindrance.

Immunoglobulin M is the principal component of primary immune response. Because of its large size (970 kDa, 19S), it is located mainly in the bloodstream. As it is not transported across the placenta, the presence of IgM in the fetus indicates intrauterine infection and its detection is useful to the diagnosis of congenital infections such as syphilis, rubella, human immunodeficiency virus (HIV) infection and toxoplasmosis. IgM antibodies are relatively short lived, disappears earlier than IgG. Hence, their demonstration in serum indicates recent infection. Treatment of serum with 0.12 M 2-mercaptoethanol selectively destroys IgM without affecting IgG antibodies.

The isohemagglutinins (anti-A, anti-B) and many other natural antibodies to microorganisms are IgM. Antibodies to typhoid O antigen (endotoxin) and Wassermann reaction (WR) antibodies in syphilis are also of this class. It is efficient in both opsonization and complement fixation.

### Immunoglobulin D

Immunoglobulin D structurally resembles IgG. The concentration is about 0.03 mg per mL of serum. It has a half-life of about 3 days. IgD acts as an antigen receptor, when present on the surface of certain B lymphocyte. Two subclasses, IgD1 and IgD2 have been described (Fig. 5.7).

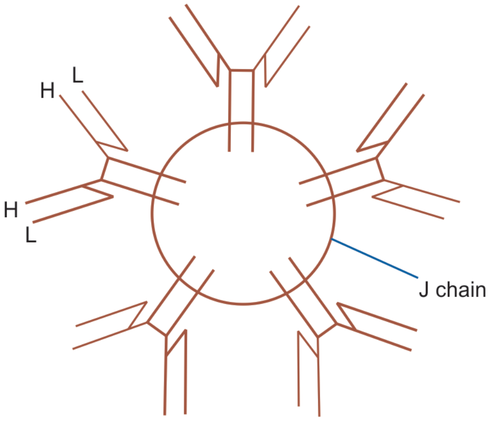


**Fig. 5.6**: Structure of human IgM (pentamer)

### Immunoglobulin E

Immunoglobulin E is 8S molecule (molecular weight is about 190,000) with a half-life of 2 days. Normal serum contains only traces. It exhibits unique properties such as heat lability and affinity towards surface of mast cells. The Fc region of IgE binds to the receptor for the antigen on the surface of mast cell and basophil. The resulting antigen-antibody complex triggers immediate (type 1) hypersensitivity reaction by releasing the mediators. Serum IgE increased in anaphylactic reaction and helminthic infection. IgE is also known as reagin (Fig. 5.8).

### Abnormal Immunoglobulins

Apart from antibodies other structurally similar proteins were seen in some pathological conditions as well as some time in healthy individuals.

Bence-Jones protein was the first abnormal protein found in multiple myeloma. The characteristic feature of this protein is that it is coagulated at 50°C, but redissolved off at 70°C. These proteins are light chains (K or L) of Ig molecules.

Myeloma is a plasma cell dyscrasia where there is unchecked proliferation of one clone of plasma cells producing one type of Ig in excessive quantity. Such Ig are called monoclonal antibodies. The myeloma may be IgG, IgA, IgD and IgE, when they involve plasma cells producing respective Igs. Involvement of IgM producing plasma cell lead to Waldenstrom macroglobulinemia.

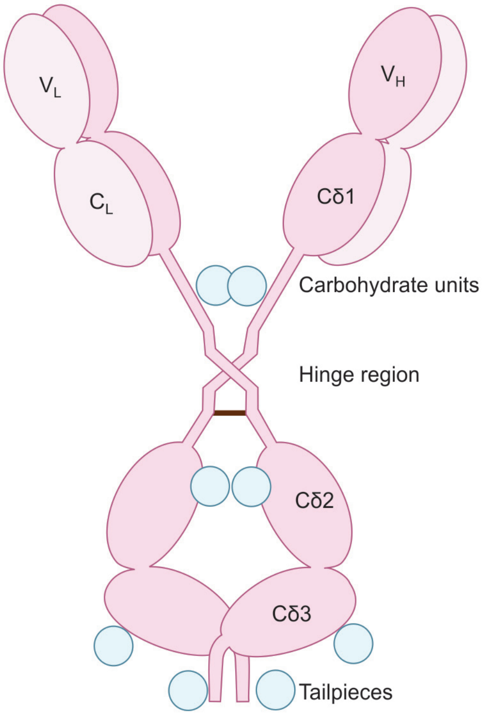


**Fig. 5.7:** Structure of IgD

## MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

### Major Histocompatibility Complex Syngeneic Human Leukocyte Antigen

*Major histocompatibility complex class I:* It consists of a heavy peptide chain of 43 kDa, non-covalently linked to a smaller 11 kDa peptide called β2-microglobulin. The part of the heavy chain is organized into three globular domains (α1, α2 and α3), which protrudes from the cell surface. The hydrophobic portion anchors the molecule on the cell membrane and a short hydrophilic sequence (arm) the COOH portion into the cytoplasm. Class I molecules are virtually present in all cells except the villous trophoblast. MHC class I molecules are the principal antigens involved in graft rejection.

*Major histocompatibility complex class II:* These are also transmembrane glycoproteins

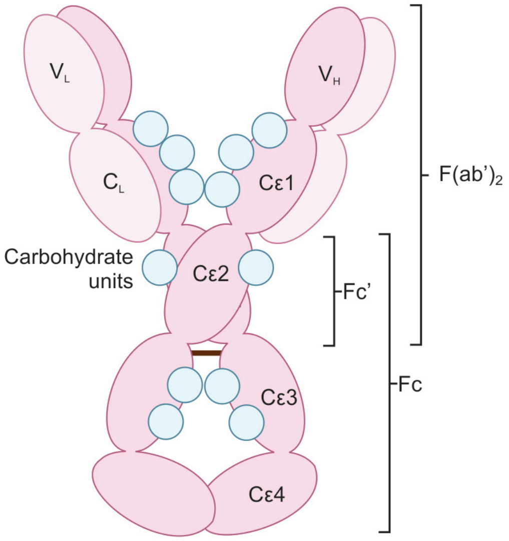


**Fig. 5.8:** Structure of IgE

consisting of α and β peptide chains of molecular weight 34 kDa and 24 kDa respectively. Both chains are folded to give two domains (α1, α2 and β1, β2). Class II molecules are particularly associated with B cells and macrophages, but can be induced on capillary endothelial cells by γ-interferons.

*T cell receptors:* The T cell receptors (TCR) complex is a combination of the antigen recognition structure (TCR) and cell activation machinery (CD3). There are two types of T cell antigen receptors, the TCR1 consisting of γ/δ chains and the TCR2 consisting of α/β chains. T cells expressing TCR1 (γ/δ cells) are more primitive T cells, generally restricted to mucosal epithelium and other tissue locations and are important for stimulating innate and mucosal immunity. TCR2 is expressed on most T cells (α/β T cells) and these cells are primarily responsible for antigen activated immune responses.

*B cell receptors:* Ig serve as B cell receptors (BCRs). B cells bear receptors that are composed of two identical H chains and two identical L chains. In addition, secondary components (Igα and Igβ) are closely associated with the primary receptor and are thought to couple it to intracellular signaling pathways (Refer Fig. 5.1).

## Immunoglobulin Specificities

Immunoglobulins are protein in nature, hence antigenic. They exhibit immunological specificities. The antigenic specificities, which distinguish the different classes and subclasses of immunoglobulins present in all normal individual of a given species are termed isospecificities.

The antigenic specificities, which distinguish the Ig of same class between different groups of individuals in the same species are called allotypic specificities. They are under genetic control. The examples are Gm ($V_H$), 25 types and Inv (KL), three types.

Antigenic specificities exclusive to each Ig molecule is known as idiotypic specificities. An idiotype is a unique antigenic determinant of the hypervariable region, produced by specific clone of antibody-producing cells. An anti-idiotypic antibody reacts with V domain of the specific Ig molecule that induced it.

## STUDY QUESTIONS

### Essay Questions

1. Name the molecules, which recognize the antigen. Describe the structure and functions of IgG.

### Short Notes

1. Secretory immunoglobulin.
2. IgM.
3. IgE.
4. MHC I molecule.
5. MHC II molecule.

### Match the Antibody Class with its Characteristics

| Characteristics | Immunoglobulin class |
|---|---|
| Crosses placental barrier | IgM |
| Millionaire molecule | IgG |
| Takes part in anaphylaxis | IgA |
| Local immunity | IgE |

## SUGGESTED READING

1. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
2. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and company; 2007.
3. Greenwood D, Slack R. Medical Microbiology. 15th edition. 1997.
4. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
5. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby: Elsevier; 2006.

6. Peakman M, Vergani D. Basic and Clinical Immunology, 1st edition; 1997.
7. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
8. Tortora, Funke, Case. Microbiology an Introduction, 6th edition; 1998.

# Antigen-antibody Reactions

# 6

A long ago in medicine, diagnosing a disease was essentially a matter of observing patient's signs and symptoms. We have seen that increasing knowledge about the specificity of the immune system led to making protective vaccines. This immunospecificity has also helped to evolve many immunodiagnostic procedures against infectious agents and non-infectious substances such as enzymes, hormones, etc. The known antigen can be used to find out specific antibody and vice versa, as both antigen and antibody reacts in an observable manner.

In the body, the antigen-antibody reaction forms the basis of humoral immunity against the infectious diseases or the tissue injury in some types of hypersensitivity reactions and autoimmune diseases. In the laboratory, the antigen-antibody reactions help in the diagnosis of infectious diseases and non-infectious diseases. They may also be helpful in the epidemiological surveys. In general, these reactions can be used in the detection and quantification of either antigens or antibodies. Antigen-antibody reactions occurring *in vitro* is known as serological reactions.

## STAGES OF ANTIGEN-ANTIBODY INTERACTIONS

The antigen-antibody interactions occur in three stages. They are:

1. Primary stage.
2. Secondary stage.
3. Tertiary stage.

### Primary Stage

No visible effect is produced. The reaction is rapid and occurs in low temperature. The reaction obeys the laws of thermodynamics and physical chemistry. The reaction is reversible being effected by weaker intermolecular forces such as van der Waals, ionic and hydrogen bonding.

### Secondary Stage

Secondary stage follows the primary stage leading to demonstrable effects such as precipitation, agglutination, lysis of the cells, immobilization, killing of the living antigen, neutralization of the toxins, etc. Unlike the primary stage, secondary stage is irreversible.

### Tertiary Stage

Some antigen-antibody reactions occurring *in vivo* initiate chain reactions that leads to neutralization or destruction of injurious antigens or to tissue damage. Tertiary reactions also include humoral immunity against infectious diseases, as well as clinical allergy and other immunological diseases.

## MEASUREMENT OF ANTIGEN AND ANTIBODY

Measurement of antigen and antibody can be done in terms of mass or more commonly as units or titers. The antibody titer of a serum is the highest dilution of the serum, which gives an observable reaction with the antigen in the particular test. Antigens also may be titrated against sera.

Sensitivity and specificity are two important parameters need to be known in any serological test. Sensitivity refers to the ability of the test to detect even very minute quantities of antibody or antigen. False negative reactions are uncommon in a highly sensitive test. Specificity refers to the ability of the test to detect reaction between homologous antigen and antibodies only. False positive reactions are absent or minimal.

## PRECIPITATION REACTIONS

Historically, one of the first serological tests to be developed was the precipitation test, which can be used to detect antigen or antibodies.

### Definition

When a soluble antigen combines with its specific antibody, in the presence of electrolyte [sodium chloride (NaCl)], at a suitable temperature and pH, the antigen-antibody complex forms insoluble precipitates.

Precipitation reactions occur in two stages. First, the antigens and antibodies rapidly form small antigen-antibody complexes. This interaction occurs within seconds and is followed by a slower reaction, which may take minutes to hours in which antigen-antibody complexes forms lattices that precipitate from solution.

When the antigen-antibody complexes instead of sedimenting remains suspended as the floccules the reaction is known as flocculation.

### Zone Phenomenon

The amount of precipitate is greatly influenced by the relative proportion of antigen and antibody. If to the same amount of antiserum in different tubes, excess of antigens are added, the precipitation will be found rapidly and abundantly in the middle tubes, where the proportion of antigen and antibody are same. The precipitations are scanty in preceding tubes (zone of antibody excess or prozone) and also in the latter tubes (zone of antigen excess or postzone). This can be plotted in a graph, the resulting curve will have three phases:

1. An ascending part (prozone).
2. Peak (zone of equivalence).
3. Descending (postzone).

The prozone phenomenon occurs, when antibody or antigen is in excess and suboptimal immune complexes form. This phenomenon may lead to misinterpretation, when large amounts of antibody are present (e.g. in multiple myeloma or polyclonal gammopathies) or when antigens are improperly diluted (Figs 6.1, 6.2A and B).

### Mechanism (Lattice Hypothesis)

Multivalent antigen combines with bivalent antibodies in varying proportion depending on the antigen-antibody ratio in the reaction mixture. Precipitation results, when large lattice is formed consisting of alternating antigen and antibody molecules. Lattice is possible in zone of equivalence.

#### *Consequences of Failure of Lattice Formation*

The formation of soluble complexes and inhibition of aggregation in antigen excess is most important in the pathogenesis of several conditions, which are classed together as immune complex diseases. Complexes, which form in the circulation are normally removed by the macrophages of the spleen and liver.

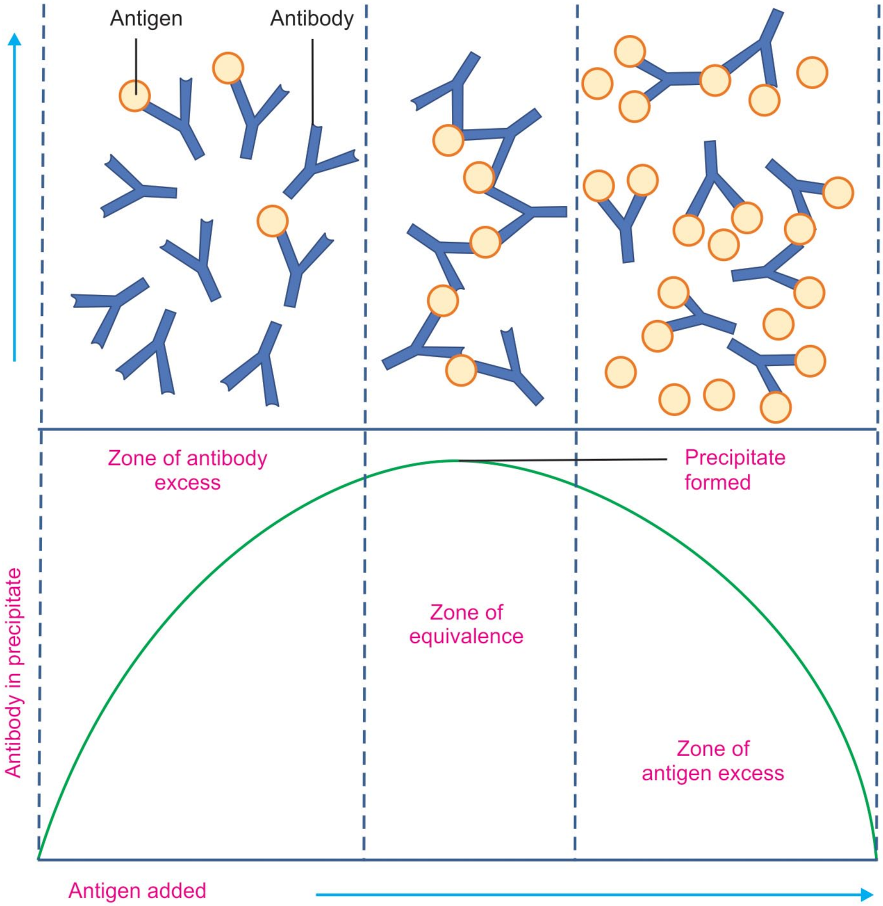

Antigen
Antibody
Zone of antibody excess
Precipitate formed
Zone of equivalence
Zone of antigen excess
Antibody in precipitate
Antigen added

**Fig. 6.1**: A precipitation curve. The curve is based on the ratio of antigen to antibody. The maximum amount of precipitate forms in the zone of equivalence where the ratio is roughly equivalent.

Complexes, which cannot be removed in this way because of their small size and excessive quantity tend to become deposited in and around the blood vessels in the kidneys, joints, heart and skin leading to immune complex diseases such as poststreptococcal glomerulonephritis, rheumatic fever, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), etc. More about immune complex diseases will be dealt in the Chapter of Hypersensitivity, Type III reaction.

## Application of Precipitation Reaction

Precipitin tests are not widely used in most laboratories. However, modifications of the precipitation of the antigen and antibodies diffusing in a solid medium with or without electric current have been popular.

Precipitation tests are mostly used to detect antigen. As less as 1 ng of protein can be detected. It is less sensitive to detect antibodies. Precipitation test may be used for identification of blood and seminal fluid (forensic application).

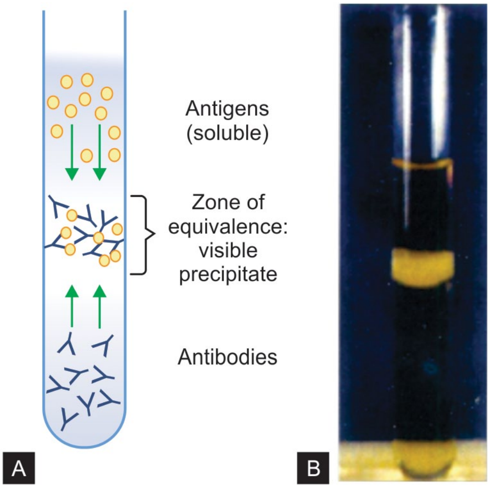
### *Precipitation in Liquid*

1. Ring test: Layering of antigen solution over a column of antiserum in a narrow tube. For example,
    - C-reactive protein (CRP)
    - Lancefield grouping of S*treptococcus*
    - Ascoli's thermoprecipitation test.
2. Slide flocculation test: Venereal disease research laboratory (VDRL) test for syphilis.
3. Tube test: The Kahn's test for syphilis is an, example for the tube flocculation test.

A quantitative flocculation test is employed for the standardization of toxins and toxoids.

### *Precipitation in Gel (Immunodiffusion)*

Precipitation is performed in 1 percent soft agar gel (Figs 6.3A and B).



**Figs 6.2A and B**: Precipitin ring test. **A.** This drawing shows the diffusion of antigens and antibodies towards each other in a small-diameter test tube. Where they reach equal proportions in the zone of equivalence, a visible line or ring of precipitate is formed; **B.** A photograph of a precipitin ring.

*Advantages*

1. The reactions visible as distinct band of precipitation, which is stable and can be stained for preservation.
2. A number of antigens can be observed, as each antigen produces line of precipitation.
3. Immunodiffusion indicates identity, cross reaction and non-identity.

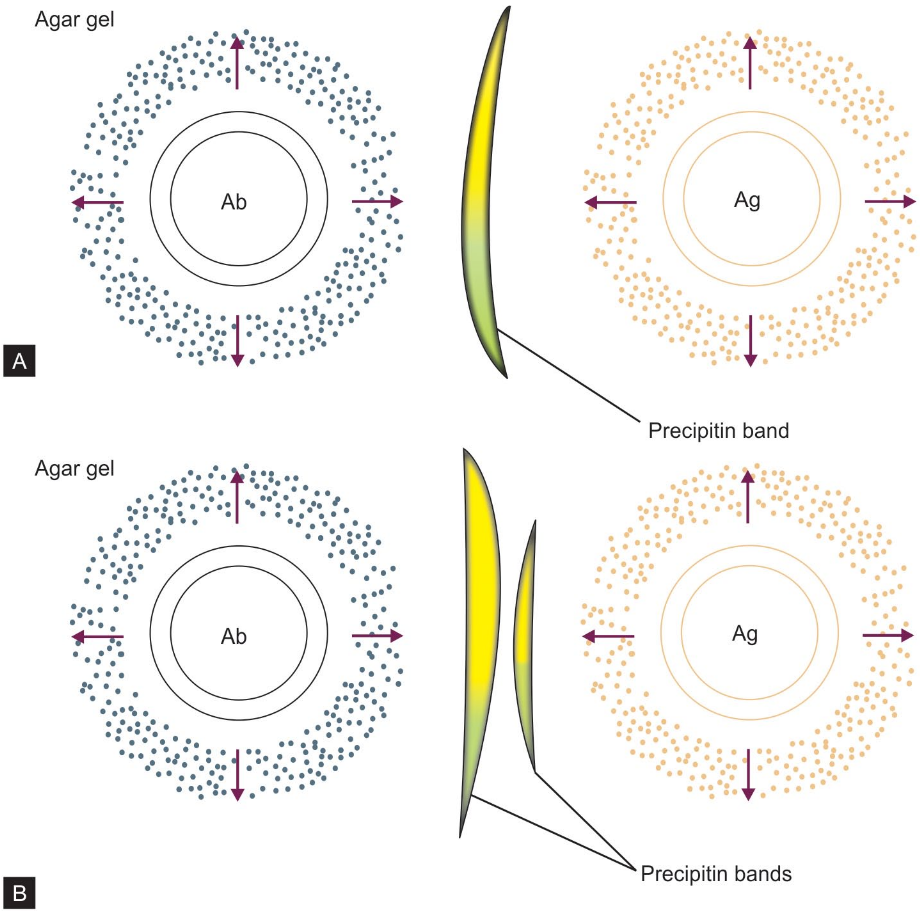
### *Modifications of Immunodiffusion Techniques*

*Single diffusion in one dimension (Oudin procedure):* The test is either done in test tube or in capillary tube. Antigen solution layered on agar gel incorporated with antibody. Antigen diffuses downwards forming a line of precipitation (Fig. 6.4).

*Double diffusion in one dimension (Oakley procedure):* It is also done in test tube or capillary tube. Antibody (serum) is incorporated in agar gel and is placed in the bottom of the tube over which plain agar is kept. Then the unknown antigen solution is poured over plain agar. Antigen and antibody moves towards each other through intervening column of plain agar and form a band of precipitation (Fig. 6.5).

*Single diffusion in two dimensions:* The antibody (antiserum) is incorporated in agar gel and poured over a slide or Petri plate. The unknown antigen is added to the well, cut on the surface of agar. Antigen diffuses radially from well and forms a ring-shaped band of precipitation. The diameter of the hollow gives the concentration of the antigen (Figs 6.6A and B). This technique is used for the quantitative estimation of antigen or antibodies.

1. Estimation of immunoglobulin in serum.
2. Screening sera for antibodies to influenza virus.
3. Determination of serum C3.
4. Estimation of transferrin.
5. Estimation of $\alpha$-fetoprotein.



**Figs 6.3A and B**: In immunodiffusion, a modification of the precipitin reaction, wells are made in an agar gel and filled with test solutions of antigen (Ag) and antibody (Ab). **A.** A single antibody and antigen diffuse outward from the wells, meet, react with each other and precipitate. They form a line called precipitin band, which is visualized by staining; **B.** Two different antigen-antibody complexes diffuse at different rates producing separate bands.

*Double diffusion in two dimension (Ouchterlony technique):* This technique is most widely employed. This helps to compare antigen and antisera directly.

1. For diagnosis of smallpox.
2. Elek test for toxigenicity for *Corynebacterium diphtheriae.*
3. Aspergillosis.
4. Farmer's lung.

This test is done in a Petri dish or microscopic slide with thin layer of clear agar (Fig. 6.7).

### *Immunoelectrophoresis*

When serum samples contain several antigens, immunoelectrophoresis can be used to detect separate antigen-antibody complexes. Resolving power of immunodiffusion is enhanced, when immunoelectrophoresis was devised. The antigens are placed in a well on an agar-coated slide. An electric current is then passed through the gel. During electrophoresis different antigen molecules migrate at different rates, depending on the size and electric charges of the molecule. Following electrophoresis, the antibody is placed in a trough made along on or both the sides of the slide and diffusion is allowed to occur.

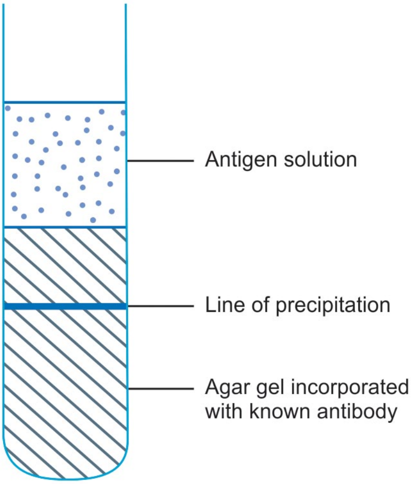

Antigen solution
Line of precipitation
Agar gel incorporated with known antibody

**Fig. 6.4:** Precipitation in gel; single diffusion in one dimension

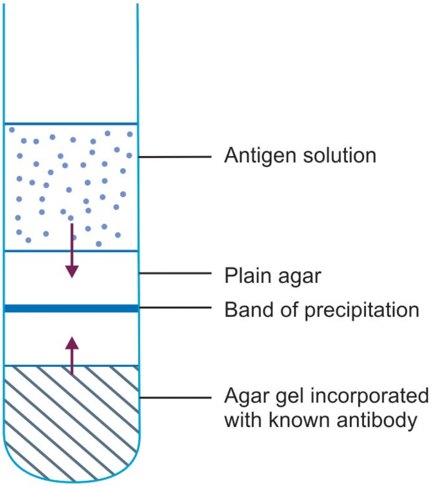

Antigen solution
Plain agar
Band of precipitation
Agar gel incorporated with known antibody

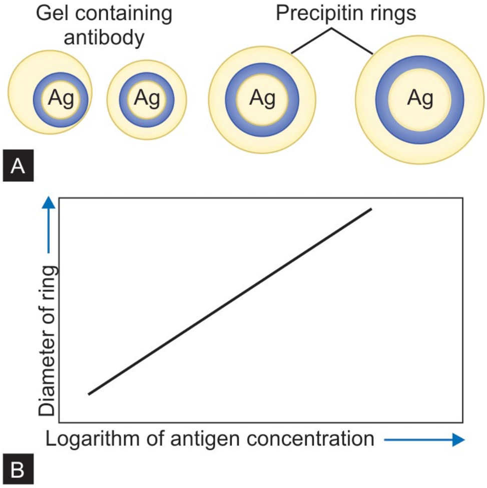
**Fig. 6.5:** Precipitation in gel; double diffusion in one dimension

The results of immunoelectrophoresis are similar to those obtained in other immune diffusion tests—precipitation bands form, whenever matching antigen-antibody precipitate (Figs 6.8A to C).

Thus, the advantage of immunoelectrophoresis is the ability to separate several antigens that might be present in a serum sample.

### *Electroimmunodiffusion*

The development of precipitate line can be speeded up by electrically driving the antigen and antibody.


Gel containing antibody
Precipitin rings
Ag
Ag
Ag
Ag
A
Diameter of ring
Logarithm of antigen concentration
B

**Figs 6.6A and B:** In radial immunodiffusion. **A.** Wells cut into antibody-containing agar sheets, which are filled with antigen. The antigen diffuses outward, complexing with antibody as it goes. When the ratio of antigen to antibody is optimal, the complexes precipitate in a ring; **B.** The diameter of the ring is proportional to the logarithm of the antigen concentration, which can be determined by reference to a standard curve. The concentration of an antibody can also be determined by placing it in a well cut into an antigen-containing agar sheet and comparing the size of the resulting ring with a standard curve for that antibody.

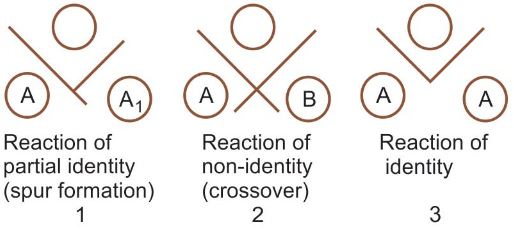

A
$A_1$
Reaction of partial identity (spur formation)
1
A
B
Reaction of non-identity (crossover)
2
A
A
Reaction of identity
3

**Fig. 6.7:** Ouchterlony technique; this figure shows the different reactions of identity.

*Countercurrent immunoelectrophoresis:* This involves simultaneous electrophoresis of antigen and antibody in gel, in opposite directions resulting precipitation, at a point between them (Fig. 6.9). This method is useful for detection of various antigens.

1. Hepatitis B surface antigen (HBsAg).
2. α-fetoprotein.
3. Cryptococcal antigen in cerebrospinal fluid (CSF).

*Rocket electrophoresis:* This method may be used for quantitative estimation of antigen.

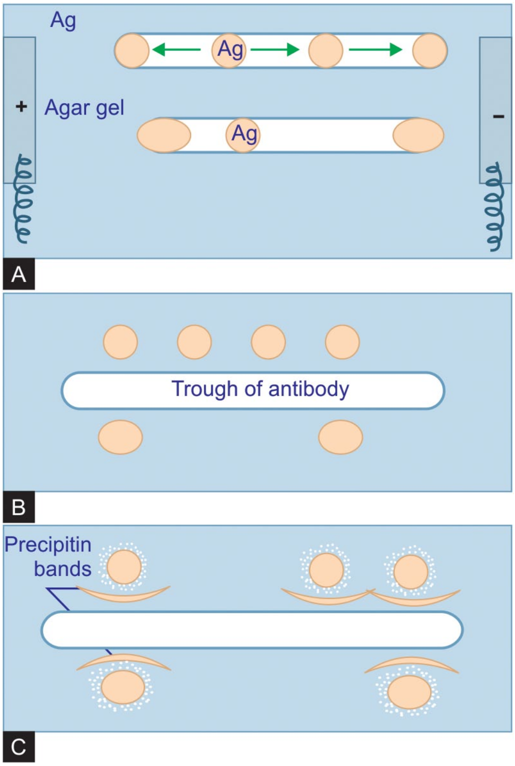
The antiserum to the antigen to be quantified is incorporated in the agar gel. The antigen, in increasing concentration, is placed in wells punched in set gel. The antigen is then electrophoresed into the antibody-containing agarose. The pattern of electrophoresis resemble a rocket and hence the name is rocket electrophoresis (Fig. 6.10).

Laurell modification is a variant of rocket electrophoresis. The antigen mixture is first electrophoretically separated. The separated antigen is electrophoresed into antibody-containing agarose.

## AGGLUTINATION REACTION

### Definition

When particulate antigens (particles such as cells that carry antigenic molecules or soluble antigens adhering to particles) combine with its homologous antibody, in the presence of an electrolyte at a suitable temperature and pH, the particles are clumped or agglutinated.



**Figs 6.8A to C:** Immunoelectrophoresis. **A.** Antigens placed in an agar gel are separated by means of an electric current. Positively charged molecules are drawn towards the negative pole, negatively charged ones move towards the positive pole; **B.** A trough is cut into the agar between the wells and filled with antibody; **C.** Curved precipitin bands form, where antigens and antibodies diffuse to meet and react.

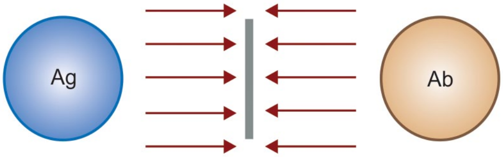


**Fig. 6.9:** Countercurrent immunoelectrophoresis (CIE); antigen (Ag) and antibody (Ab) are driven together by an electric current and a precipitin line forms.

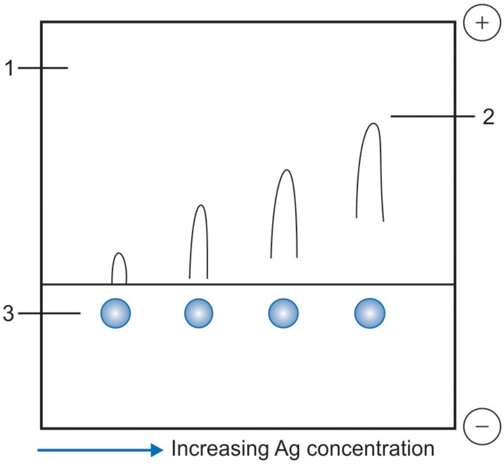


**Fig. 6.10:** Rocket electrophoresis. **1.** Antibody in agar gel; **2.** Precipitin arcs; **3.** Antigen (Ag) wells.

Agglutination reactions are very sensitive, relatively easy to read and available in great variety.

Zone phenomenon may be seen either in antigen excess or antibody excess. Agglutination occurs optimally, when antigens and antibodies react in equivalent proportion.

## Mechanism

The same lattice hypothesis holds good. The antigenic determinants on the surface of the cell or of an inert particle coated with (soluble) antigen are linked together by the multivalent antibody (Fig. 6.11).

Incomplete or monovalent antibodies do not cause agglutination though combined with antigen.

## Application of Agglutination Reaction

### Direct Agglutination Test

Direct agglutination test detects antibodies against relatively large cellular antigens such as those on the red blood cells (RBCs), bacteria and fungi (Figs 6.12A and B).

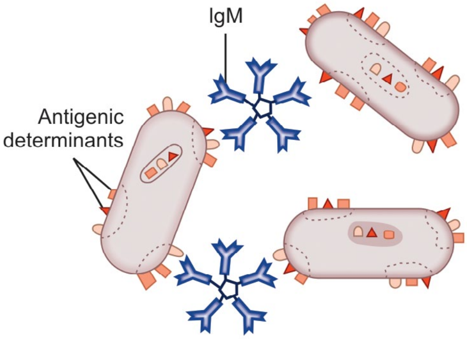


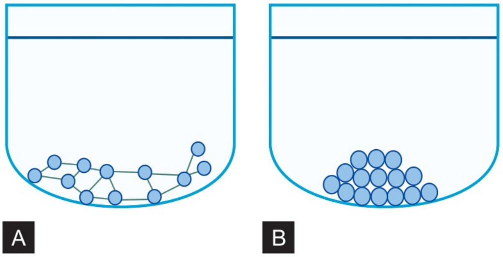
**Fig. 6.11:** An agglutination reaction when antibodies react with antigenic determinant sites on antigens carried on neighboring cells, such as these bacteria (or red blood cells). The particulate antigens (cells) agglutinate. IgM, the most efficient immunoglobulin for agglutination is shown here, but IgG also participates in agglutination reactions.

*Slide agglutination:* It is of three types.

1. Bacterial agglutination: Antiserum is added on a uniform suspension of pure bacteria. If there is clumping as well as clearing of the suspension, then the agglutination is positive. Control should be done along with the test with normal saline. Slide agglutination is routine procedure for the identification of bacterial isolates from clinical specimen.
2. Red cell agglutination: It is also the method used for blood grouping and cross matching.
3. Widal's slide agglutination test.

*Tube agglutination:* This is a standard quantitative method for the measurement of antibodies. Tube agglutination is routinely employed for the diagnosis of enteric fever, brucellosis, typhus fever, infectious mononucleosis and many other conditions.

Widal's test is used for the diagnosis of enteric fever by *Salmonella (S. typhi, S. paratyphi A* and *S. paratyphi B).* Two types of antigens,



**Figs 6.12A and B:** Direct agglutination test showing positive and negative reaction. **A.** In a positive (agglutination) reaction, sufficient antibodies are present in the serum to link the antigens together forming a mat of antigen-antibody complexes on the bottom of the well; **B.** In a negative (no agglutination) reaction, not enough antibodies are present to cause the linking of antigens. The particulate antigens roll down the sloping sides of the well, forming a pellet at the bottom.

H for flagellar and O for somatic are used to detect for the presence of antibodies in the patient's serum. The antigens are formalized suspension of bacilli and on combination with H antibody form large, loose fluffy clumps resembling wisp of cotton wool. Dreyer tubes are used for agglutination. The O antigen is prepared by treating the bacterial suspension with alcohol. It forms tight compact deposits resembling chalk powder. For demonstration of O agglutinins, round bottom Felix tubes are used. For diagnosis of enteric fever, not only a diagnostic titer (for H 200 and for O 100) is required, but also an increasing titer in subsequent tests is required. That means, the quantity of antibodies against particular infectious agent in patient's blood is increasing. The quantity of antibodies is called antibody titer. It is reported as the reciprocal of the greatest serum dilution in which agglutination occurs. For example, an antiserum that agglutinated *S. typhi* antigen at a dilution of 1:256, but not at 1:512 would be reported as antibody titer of 256. The production of antibodies in the serum resulting from the infection is called seroconversion. The tube agglutination test measures antibody titers by comparing various dilutions of patient's serum against the same known quantity of the antigen.

The tube agglutination test for brucellosis may be complicated by the prozone phenomenon and the presence of blocking antibodies. Several dilutions of the serum should be tested to prevent false negative reaction. Incomplete or blocking antibodies may be detected by antiglobulin (Coombs') test.

The Weil-Felix reaction for serodiagnosis of typhus fever is a heterophile agglutination test, where *Proteus* strains (OX-19, OX-2 and OX-K) are used as antigens.

Paul-Bunnell test is also done for the diagnosis of infectious mononucleosis, which uses sheep red cells as antigen, which probably shares the antigenic similarity with Epstein-Barr virus (EBV).

Other tube agglutination test such as *Streptococcus* MG agglutination test based on heterophile antibody is done for the diagnosis of primary atypical pneumonia, where the suspected etiological agent is *M. pneumoniae.*

In cold agglutination test, the patient's sera agglutinate human O-group erythrocytes at 4°C. The agglutination is reversible at 37°C.

### *Indirect (Passive) Agglutination Test*

Antibodies against soluble antigens can be detected by agglutination tests, if the antigens are adsorbed onto particles such as bentonite or latex particles. Such tests known as latex agglutination tests are commonly used for the rapid detection of the serum antibodies against bacterial and viral diseases. In such indirect (passive) agglutination tests, the antibody reacts with soluble antigen adhering to the particles. The particles then agglutinate with one another.

The same principle can be applied in reverse by using particles coated with antibodies, to detect the antigens against which they are specific (e.g. streptococci in sore throat).

Latex agglutination tests both passive and reverse passive are widely employed in the clinical laboratory (HBsAg, ASO, CRP, RA factor, classic pregnancy test, etc.) (Figs 6.13A and B).

### *Hemagglutination*

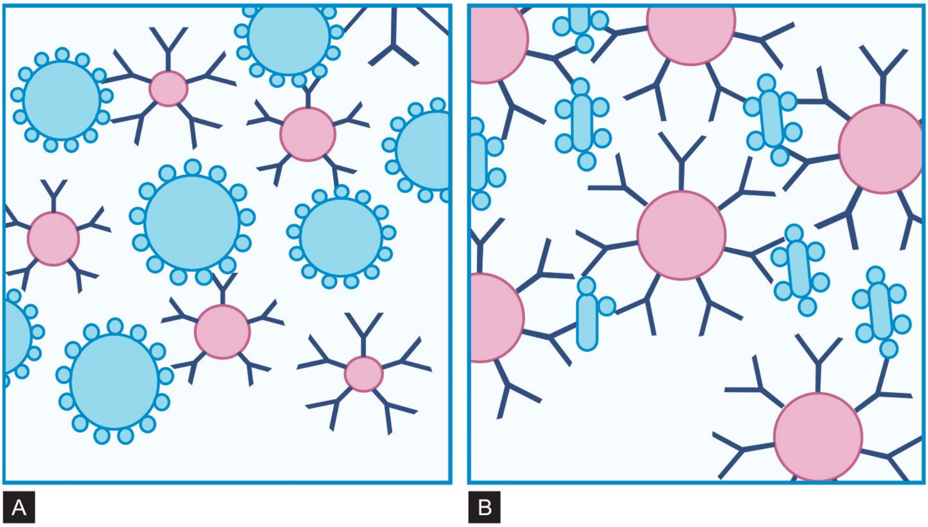
When agglutination reaction involve the clumping of the RBCs, the reaction is called hemagglutination (Fig. 6.14). These reactions, which involve RBCs surface antigens and their complementary antibodies are used routinely in blood typing and in the diagnosis of infectious mononucleosis.

Certain viruses such as those causing mumps, measles and influenza, have the ability to agglutinate RBCs without an antigen-antibody reaction; this process is called viral hemagglutination (Refer Fig. 6.14). This type of hemagglutination can be used to detect antibodies that neutralize the agglutinating viruses in patient's sera.

### *Coombs' Test*

Coombs' test is devised by Coombs, Mourant and Race (1945) for detection of incomplete anti-Rh antibodies that do not agglutinate Rh-positive erythrocytes in saline.

*Principle:* When sera containing anti-Rh antibodies is mixed with Rh-positive erythrocytes in saline, antiglobulin coats over the surface, but they are not agglutinated. Although anti-Rh antibodies will bind to Rh antigen on RBCs, there are not sufficient antigens to cause clumping. When such erythrocytes coated with antibody globulin are treated with a rabbit antiserum against human globulin (antiglobulin or Coombs' serum), the antibody-cell complexes will agglutinate (Figs 6.15A and B).



**Figs 6.13A and B:** Reaction in indirect agglutination tests. These tests are performed using antigens or antibodies coated onto particles such as minute latex spheres. **A.** When particles are coated with antigens, agglutination indicates the presence of antibodies such as the IgM shown here; **B.** When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

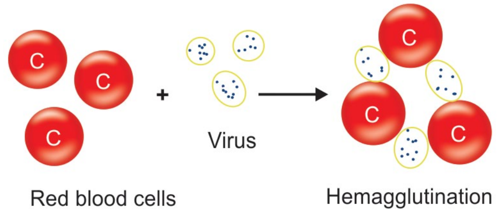


**Fig. 6.14**: Viral hemagglutination

*Types: Direct Coombs' test*: When one drop of Coombs' serum is added to washed RBCs from an infant of erythroblastosis fetalis, agglutination results; the test is done to know the presence of anti-Rh antibodies on the red cells of the infant suffering from erythroblastosis fetalis.

*Indirect Coombs' test:* Serum of sensitized mother is tested for the presence of anti-Rh antibodies. A drop of serum from sensitized mother is mixed with washed O-group red cells. Subsequently one drop of Coombs' serum (antiglobulin serum) is added. The RBCs agglutinate. Coombs' test is also useful for demonstrating any type of incomplete or non-agglutinating antibody as for example, in brucellosis.

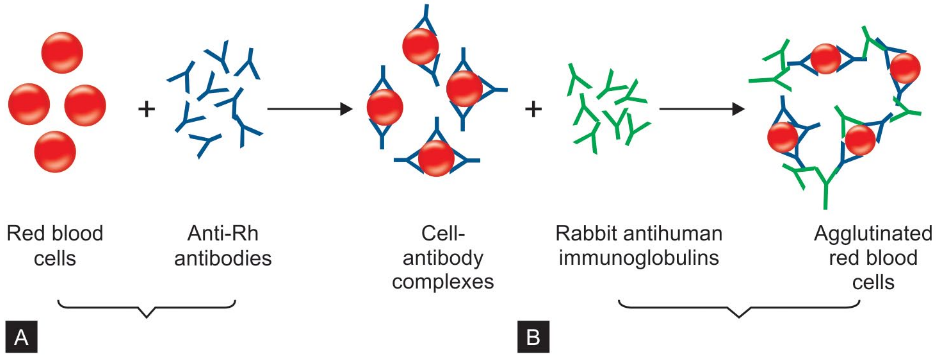
*Rose-Waaler Test*

Rose-Waaler test is a special type of passive hemagglutination test, which detects rheumatoid factor (RA factor) in the serum of the patient suffering from RA. RA factor is the antibody to IgG.

## Coagglutination

An effective and adaptive modification of the principle of agglutination has been developed, making use of separate binding sites on the IgG molecule.

Immunoglobulin binds to the protein A of *Staphylococcus aureus* (Cowan-1 strain) by the fraction crystallizable (Fc) terminal portion, leaving the antigen combining fragment antigen-binding (Fab) terminal free. Thus, *Staphylococcus* coated with IgG antiserum can be used in agglutination tests for bacterial antigens (Figs 6.16A and B). The test has been used for streptococcal grouping, as well as the typing of *Neisseria gonorrhoeae*, mycobacterial grouping and for identifying antibacterial antibodies and antigens directly in body fluids.


+
+
Red blood cells
Anti-Rh antibodies
Cell-antibody complexes
Rabbit antihuman immunoglobulins
Agglutinated red blood cells
A
B

**Figs 6.15A and B**: Coombs' antiglobulin test. **A.** Anti-Rh antibodies are allowed to react with red blood cells. If Rh antigens are present on the blood cells, there are not enough of them to produce a hemagglutination reaction; **B.** Therefore, antihuman antibodies prepared in rabbits are reacted with the red blood cell-antibody complexes. If Rh antigens are present on the red blood cells, hemagglutination will occur. A person with these red blood cells would be Rh positive.

## Opsonization

Opsonins are the substances (complement and antibody, IgM), which coats on the target cells and make them palatable for phagocytosis. The process initiated by the opsonins is called opsonization or immune adherence.

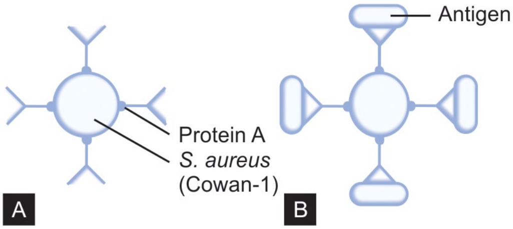
1. Phagocytes have some intrinsic ability to bind directly to bacteria and other microorganisms, but this is much enhanced if the bacteria have activated complement.
2. They will then have bound C3b, so that the cells can bind the bacteria via C3 receptors.
3. Organisms, which do not activate complement well if at all, are opsonized by antibody (Ab), which can bind to Fc receptors on phagocyte.
4. Antibody can also activate complement and if both antibody and C3b opsonize the microbe, the binding is greatly enhanced (Fig. 6.17).

Opsonin index is defined as the ratio of the phagocytic activity of the patient's blood for a given bacterium to the phagocytic activity of the blood from a normal individual.

## Neutralization

### *Virus Neutralization*

Virus neutralization can be demonstrated in various systems. Neutralization of bacteriophage can be demonstrated in plaque inhibition. When the bacteriophages are seeded in appropriate dilution on lawn cultures of susceptible bacteria, plaque lysis occur. Specific antiphage serum inhibits plaque formation.



**Figs 6.16A and B:** Coagglutination. **A.** Antibody-coated *S. aureus* (Cowan-1 strain, rich in protein A); **B.** *S. aureus* captures desired antigen and form lattice.

Antibodies may effect virus neutralization by several mechanisms. They may prevent virus adsorption to cell receptors. A more frequently used neutralization test is the viral hemagglutination inhibition (HI) test. This test is used in the diagnosis of influenza, measles, mumps and a number of other infections caused by viruses that can agglutinate RBCs. If a person's serum contains antibodies against these viruses, the antibodies will react with viruses and neutralize them (Fig. 6.18).

### *Toxin Neutralization*

Bacterial exotoxins are good antigens and induce antibody (antitoxin) formation. These antitoxins, for example, diphtheria and tetanus, are useful in the treatment of diphtheria and tetanus (Fig. 6.19). The toxin-antitoxin complex is ingested by macrophage and eliminated.

Toxin neutralization may occur *in vivo* (Schick test) and is based on the ability of the circulating antitoxin to neutralize the diphtherial toxins injected intradermally. This test indicates susceptibility or immunity. An example of toxin neutralization *in vitro* is the antistreptolysin O titer test. In this test, the antitoxin present in patient's sera neutralize the hemolytic activity of the streptococcal O hemolysin.

## Complement-mediated Serological Reactions

A group of serum proteins present in normal serum are collectively called complement. Complement takes part in many immunological reactions and is absorbed during combination of antigens with their antibodies. In the presence of appropriate antibody, complement lyses erythrocytes, kills bacteria, immobilizes motile organisms, promotes phagocytosis and immune adherence and

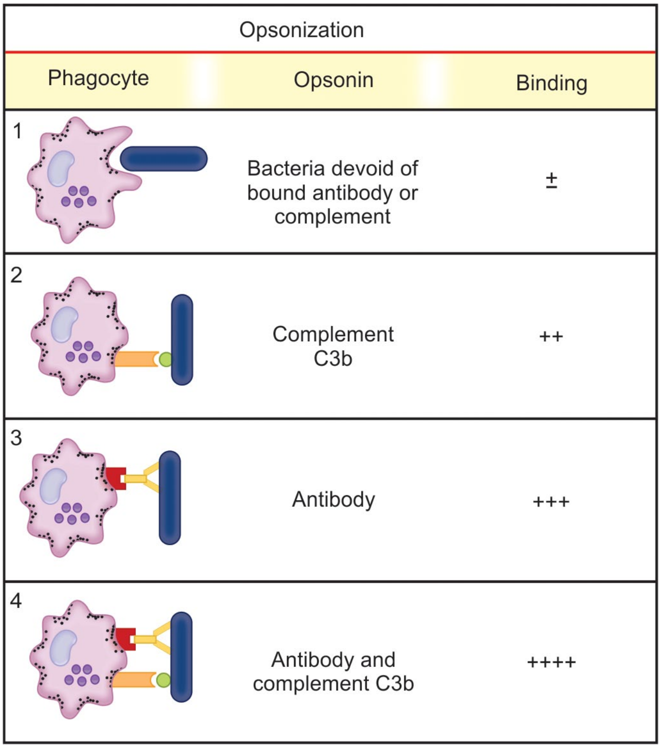

Opsonization
Phagocyte
Opsonin
Binding
1
Bacteria devoid of bound antibody or complement
±
2
Complement C3b
++
3
Antibody
+++
4
Antibody and complement C3b
++++

**Fig. 6.17:** Opsonization. **1.** Phagocytes have some intrinsic ability to bind directly to bacteria and other microorganisms, but this is much enhanced if the bacteria have activated complement; **2.** They will then have bound C3b, so that the cells can bind the bacteria via C3b receptors; **3.** Organisms, which do not activate complement well, if at all, are opsonized by antibody (Ab), which can bind to the Fc receptor on the phagocyte; **4.** Antibody can also activate complement and if both antibody and C3b opsonize the microbe binding is greatly enhanced.

contributes to the tissue damage by certain types of hypersensitive reactions.

The complement-mediated serological reactions are:

1. Complement fixation test.
2. *Treponema pallidum* immobilization (TPI) test.
3. Immune adherence.
4. Cytolytic or cytocidal test.

### *Complement Fixation Test*

Antibodies that do not produce a visible reaction, such as precipitation or agglutination can be demonstrated by the fixing of

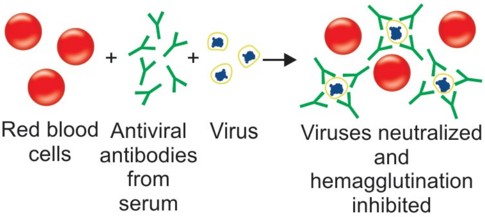

Red blood cells
+
Antiviral antibodies from serum
+
Virus
Viruses neutralized and hemagglutination inhibited

**Fig. 6.18:** Reactions in neutralization tests. In the viral hemagglutination inhibition test, serum that may contain antibodies to a virus is mixed with red blood cells and the virus. If antibodies are present, as shown here, they neutralize the virus and inhibit hemagglutination.

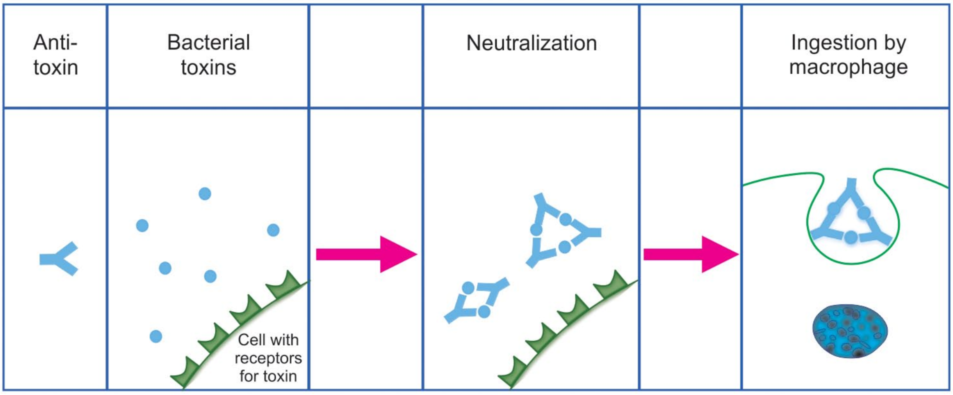


**Fig. 6.19:** Neutralization of toxins by IgA or IgG

complement during the antigen-antibody reaction. Complement fixation was once used for the diagnosis of syphilis (Wassermann test) and is still used to diagnose bacterial diseases, as pertussis and gonorrhea and fungal diseases including histoplasmosis and certain viral and rickettsial diseases. The complement fixation test can be used to detect very small amount of antibodies. If an antibody-antigen reaction takes place in the presence of a limited amount of complement, the latter is activated and thereby consumed.

An indicator system is then added to detect the presence of remaining complement. The indicator system consists of sheep red cells coated with anti-sheep red cells antibody (IgM) at a level insufficient to cause agglutination, but which cause the red cells to lyse, if complement is present. If the complement has been used up (fixed) by the reaction of antibody and antigen, no lysis of red cells occur (positive). If the antibody is not specific to the known antigen, the complement will not be used up and hence would react with indicator system (sheep red cells coated with anti-sheep red cells antibody produced in rabbit) leading to lysis (negative).

In any test system, the serum is to be decomplemented first and a non-human source of complement (guinea pig serum rich in complement) may be used. The guinea pig serum should be titrated for complement activity and minimum hemolytic dose should be found out. The minimum hemolytic dose (MHD) of complement is defined as the highest dilution of guinea pig serum that lyses one unit volume of washed sheep red cells in the presence of antibody (amboceptor) within a fixed time (30 minute) and at 37°C.

Execution of the complement fixation test requires great care and good control, one reason the trend is to replace it with newer, simpler tests. The test is performed in two stages, the complement fixation and addition of indicator system (Fig. 6.20).

### *Treponema pallidum Immobilization Test*

The TPI test used *T. pallidum* (Nichols strain), grown in rabbit testes as the antigen and was based on the ability of patient's antibody and complement, to immobilize living treponemes as observed by dark field microscopy.

### *Immune Adherence*

When *Vibrio cholerae, T. pallidum* react with specific antibody in the presence of complement and particulate material such as

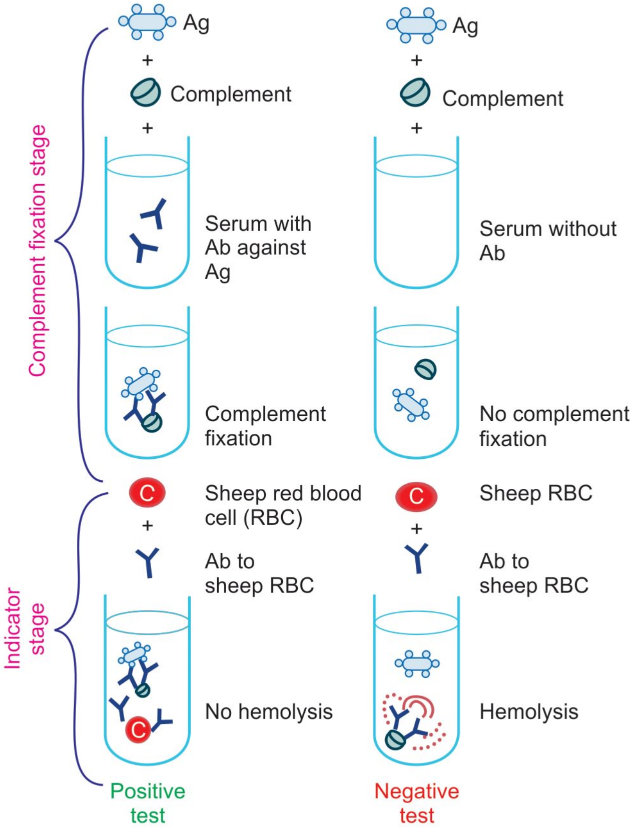

Ag
+
Complement
+
Serum with Ab against Ag
Complement fixation
Sheep red blood cell (RBC)
+
Ab to sheep RBC
No hemolysis
Positive test
Ag
+
Complement
+
Serum without Ab
No complement fixation
Sheep RBC
+
Ab to sheep RBC
Hemolysis
Negative test
Complement fixation stage
Indicator stage
C

**Fig. 6.20:** Complement fixation test

erythrocytes or platelets, the bacteria are aggregated and adhere to the cells. This is known as immune adherence. The immune adherence facilitates phagocytosis.

### *Cytolytic or Cytocidal Test*

When a suitable live bacterium such as *V. cholerae* is mixed with its antibody in the presence of complement, the bacterium is killed and lysed.

## Immunofluorescence

Fluorescent dyes [fluorescein, rhodamine, fluorescein isothiocyanate (FITC)] can be covalently attached to antibody molecules and made visible by ultraviolet light in the fluorescent microscope. Coons and his colleagues (1942) showed that the fluorescent dyes can be conjugated to antibodies and that such labeled antibodies can be used to locate and identify antigen in the tissues and clinical materials.

Fluorescent antibody technique has several diagnostic and research applications.

### *Direct Immunofluorescence*

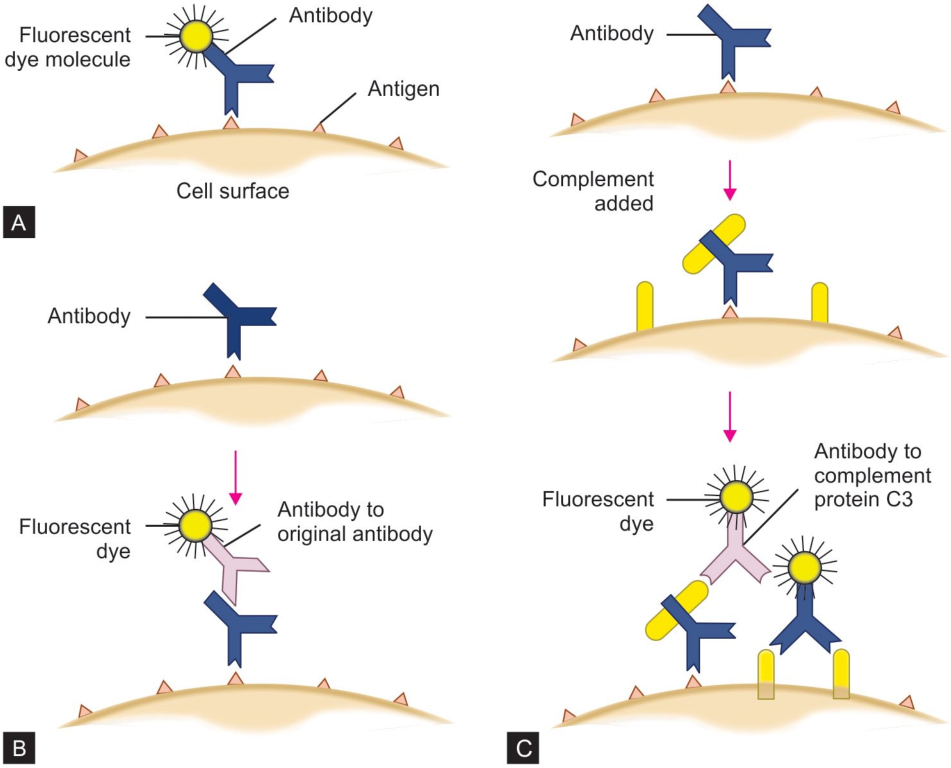
In its simplest form it is used for identification of bacteria, viruses or other antigens using the specific antiserum labeled with fluorescent dye. This is routinely used as a sensitive method of diagnosing rabies by detection of rabies virus antigen in brain smear. This can also be used for detection of gonococci, streptococci, *T. pallidum*, etc. from the smear (Figs 6.21A to C).

The disadvantage in direct immunofluorescent technique is that separate fluorescent conjugates have to be prepared for each case.

### *Indirect Immunofluorescence*

Alternatively, this basic process can be used to detect antibodies within patient sera that are reactive to specific pathogen or that are cross reactive to specific tissue antigens.

This reaction occurs when a two stage process is used for example, known antigen is attached to the slide, unknown serum is added and the preparation is washed. If the unknown serum antibody matches the antigen, it will remain fixed to it on the slide and can be detected by adding a fluorescent-


Fluorescent dye molecule
Antibody
Antigen
Cell surface
A
Antibody
Fluorescent dye
Antibody to original antibody
B
Antibody
Complement added
Fluorescent dye
Antibody to complement protein C3
C

**Figs 6.21A to C**: Immunofluorescence: Fluorescein is a fluorescent dye molecule that can be complexed with other molecules. When viewed with ultraviolet light (UV) under a fluorescence microscope it will fluoresce, revealing the presence of the 'tagged' molecule. To detect directly the presence of a specific antigen in a tissue. **A.** A solution of fluorescein-tagged antibody to that antigen is prepared, added to cells or to a thin section of tissue, incubated and then washed. Any dye-tagged antibody that has complexed with antigen in tissue will fluoresce when viewed by fluorescence microscopy; **B.** In indirect testing, the antibody to the antigen being sought is not itself tagged. Instead, its presence is detected by means of fluorescein-tagged antibody (anti-antibody) to the original antibody; **C.** Complement (protein C3) can be added to the tissue section along with the antibody and a fluorescein-tagged antibody to one of the complement proteins can then be used to detect the presence of antigen-antibody complexes or complement attached to cells.

labeled antiglobulin antibody and examining the slide by ultraviolet microscopy.

This technique is more sensitive than the direct immunofluorescence, because more labeled antibody adheres per antigenic site. More over the labeled antiglobulin becomes a universal reagent (independent of the nature of the antigen used).

Common examples are fluorescent antibody tests for syphilis, amebiasis, toxoplasmosis and many other bacterial and viral diseases (EB virus, herpes virus, etc).

Fluorescent dyes may also be conjugated with complement. Labeled complement is a versatile tool and can be employed for the detection of antigen or antibody. Antibodies to complement can also be tagged with fluorescent dyes to detect immune complex in the circulation and tissue (Refer Fig. 6.21A to C).

## Radioimmunoassay

Radioimmunoassay (RIA) is one of the most sensitive methods for measuring the concentration of antigen and antibody. RIA is widely used in the measurements of hormones, enzymes, vitamins, serum proteins, HBsAg drugs, etc. as low as 0.001 mg/mL or less can be estimated. This technique was first developed by SA Berson and Rosalyn Yalow in 1960. In 1977, some years after the death of Berson, Yalow was awarded Nobel prize in recognition to her work.

The disadvantages of RIA lie in the cost of equipments and reagents, short self-life of radiolabeled compounds and in the disposal of radioactive wastes. RIA is virtually replaced by quantitative fluorescence assays or enzyme immunoassays (EIAs).

The classical RIA methods are based on the principle of competitive binding. The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody. There are two types of RIA:

1. Soluble-phase RIA.
2. Solid-phase RIA.

### *Soluble-phase Radioimmunoassay*

The antigen is generally labeled with gamma emitting isotope such as $^{125}$I. The radiolabeled antigen is a part of the assay mixture. The test sample may be a complex mixture, such as serum or other body fluids that contain the unlabeled antigen. The first step in setting up RIA is to determine the amount of antibody needed to bind 60% to 70% of a fixed quantity of radioactive antigen (Ag*) in the assay mixture. This ratio of antibody to antigen (Ag*) is chosen to be sure that the number of epitopes to be presented by the labeled antigen (Ag*) always exceeds the total number of binding sites in antibody. Consequently, unlabeled antigen (test sample) added to the sample mixture (Ab + Ag*) will compete with radiolabeled antigen (Ag*) for the limited supply of antibody. Even if a small amount of unlabeled antigen is added to the assay mixture (labeled antigen + antibody), it will cause decrease in the amount of radioactive antigen bound and this decrease will be proportional to the amount of unlabeled antigen added. To determine bound labeled antigen, the antigen-antibody complex is precipitated to separate it from free antigen (Ag not bound to antibody). A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample) and from this plot, the amount of antigen in the text mixture may be precisely determined.

### *Solid-phase Radioimmunoassay*

Various solid-phase RIAs have been developed that make easier to separate antigen-antibody complex.

In one approach, the antigen can be immobilized in polystyrene or polyvinyl chloride wells and the amount of antibody in the test serum can be determined in a gamma counter by using radiolabeled anti-IgG (Fig. 6.22A).

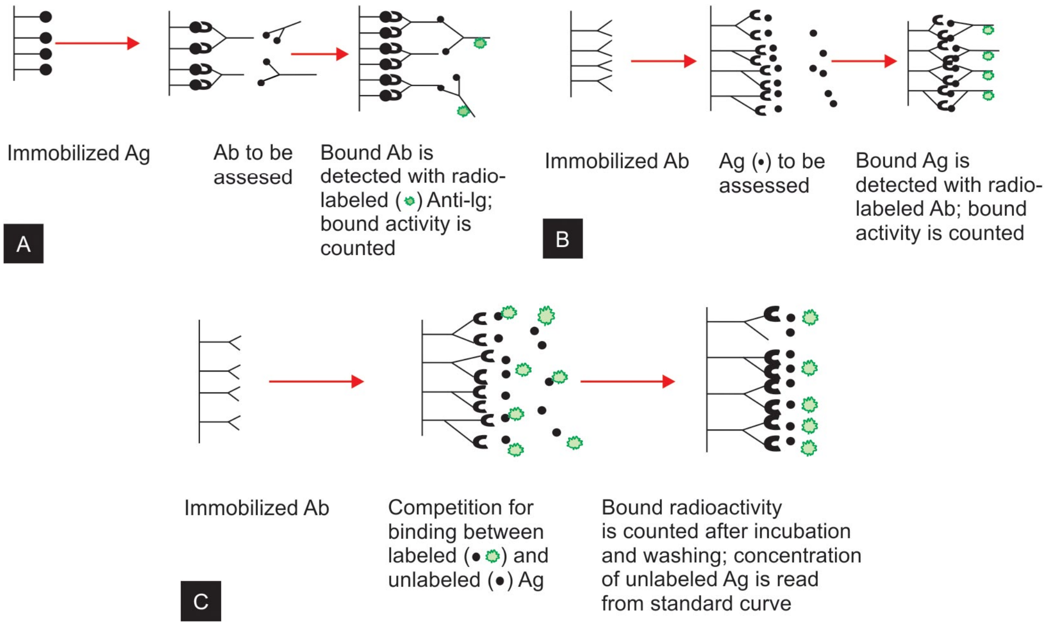
In an alternative approach, the antibody is immobilized on the walls of the microtiter wells and the amount of bound test antigen can be determined by using radiolabeled specific antibody. As this procedure requires only small amounts of sample and can be conducted in small 96-well microtiter plates, this process is best suited for determining the concentration of particular antigen (e.g. HBsAg) in large number of samples. RIA screening has been widely used to screen for the presence of the hepatitis B virus (Fig. 6.22B).

Yet in another approach (competitive binding RIA), the antibody is coupled to an insoluble support then labeled and unlabeled (test) antigens are added. After incubation and washing, bound radioactivity is counted. The more unlabeled antigen is present (bound), the less radioactive antigen will be bound. The system is first calibrated and a standard curve constructed with known amount of labeled antigen (Fig. 6.22C).

## Enzyme Immunoassay

Enzyme-labeled conjugates were introduced first in 1966 for localization of antigen in the tissue, as an alternative for fluorescent conjugates. In 1971, enzyme-labeled antigens and antibodies were developed as serological reagents for assay of antibodies and antigens.

The enzyme immunoassay is most widely used procedure in clinical serology because of its versatility, sensitivity, simplicity, cost-effectiveness and absence of radiation hazards. EIA includes all assays based on the measurement of enzyme-labeled antigen, hapten or antibody. There are two types of EIA, homogeneous and heterogeneous. In homogeneous EIA, there is no need to separate the bound and free fraction so that the test can


Immobilized Ag
Ab to be assesed
Bound Ab is detected with radio-labeled (✱) Anti-Ig; bound activity is counted
A
Immobilized Ab
Ag (•) to be assessed
Bound Ag is detected with radio-labeled Ab; bound activity is counted
B
Immobilized Ab
Competition for binding between labeled (•✱) and unlabeled (•) Ag
Bound radioactivity is counted after incubation and washing; concentration of unlabeled Ag is read from standard curve
C

**Figs 6.22A to C:** Solid phase primary binding radioimmunoassay (RIA). **A.** To assess antibody (Ab); **B.** To assess antigen (Ag); **C.** Competitive binding RIA.

be completed in one step with all reagents added simultaneously. Heterogeneous EIA requires separation of free and bound fraction by centrifugation or absorption on solid surface and then washing. It is therefore a multistep procedure [e.g. enzyme-linked immunosorbent assay (ELISA)].

### *Enzyme-linked Immunosorbent Assay*

Enzyme-linked immunosorbent assay is so named because the technique involves the use of an immunosorbent, an adsorbing material specific for one of the components of reaction, the antigen or antibody.

There are two basic methods. They are:

1. Direct (sandwich) ELISA, which can detect and quantify antigen.
2. Indirect ELISA, which can detect and quantify antibody. A microtiter plate with 96 shallow wells is used for direct and indirect ELISA (Figs 6.23A and B). Variation of the test exists; for example, the reagents can be bound to tiny latex particles rather than to the surface of the microtiter plates. ELISA procedures are popular because they primarily require little interpretive skill to read; they tend to be either clearly positive or clearly negative.

*Direct ELISA:* In the first step of the method:

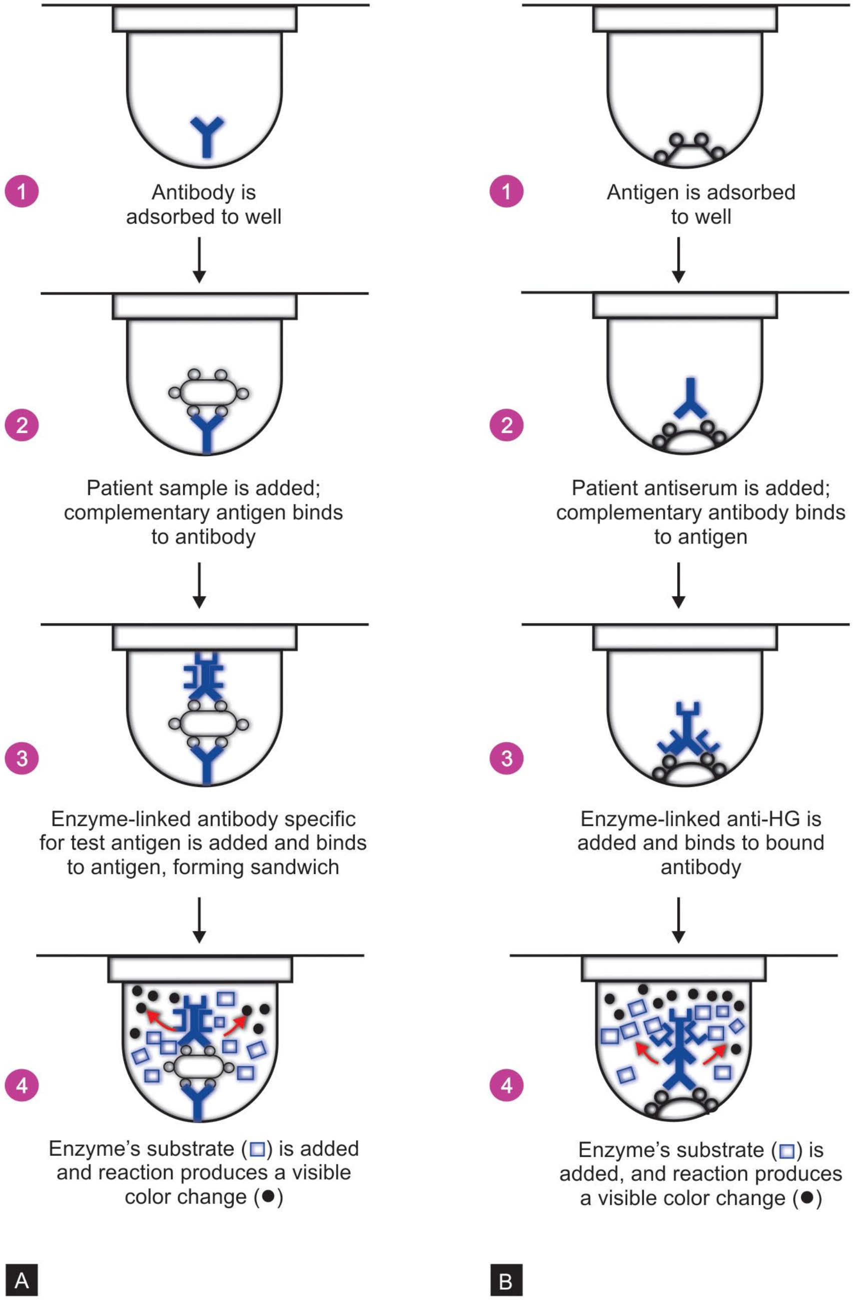
1. The antibody specific for the antigen to be detected is adsorbed to the surface of the well of the microtiter plate.
2. A patient's sample containing unidentified antigen is then added to each well. If the antigen reacts specifically with the antibodies adsorbed to the well, the antigen will be retained there when the well is washed free of unbound antigen.
3. A second antibody specific for the antigen is then added. If both the antibody adsorbed to the wall of the well and the antibody known to be specific for the antigen have reacted with the antigen, a sandwich will be formed with the antigen between two antibody molecules. This reaction is visible only because the second added antibody is linked to enzymes such as horseradish peroxidase or alkaline phosphatase. Unbound enzyme-linked antibody is washed from the well.
4. Then the enzyme's substrate is added to it. Enzymatic activity is indicated by a color change that can be visually detected. The test will be positive, if the antigen has reacted with adsorbed antibodies in the first step (Refer Fig. 6.23A).

If the test antigen was not specific for the antibody adsorbed to the wall of the well, the test will be negative because the unbound antigen would have been washed away.

*Indirect ELISA:* In the first step of this method;

1. A known antigen instead of antibody is adsorbed to the walls of the shallow well on the plate to see whether a serum sample contains antibodies against this antigen.
2. The patient's serum sample is added to the well if the serum contains antibody specific to the antigen, the antibody will bind to the adsorbed antigen. All unreacted antibodies are washed from the well. Anti-antibody (antihuman globulin) tagged with an enzyme is then allowed to react with the antigen-antibody complex.
3. Finally all unbound anti-antibody is rinsed away and the correct substrate for the enzyme is added. A colored enzymatic reaction occur in the well in which bound antigen has combined with the antibody in the serum sample (Refer Fig. 6.23B).

*Competitive ELISA:* This is another variation for measuring amounts of antigen. In this method, the known antibody (Ab1) is first incubated in solution with a sample containing antigen. The mixture (Ag + Ab) is then added to an antigen-coated well. The more antigen present in the sample the less free antibody


1
Antibody is adsorbed to well
2
Patient sample is added; complementary antigen binds to antibody
3
Enzyme-linked antibody specific for test antigen is added and binds to antigen, forming sandwich
4
Enzyme's substrate (□) is added and reaction produces a visible color change (●)
A
1
Antigen is adsorbed to well
2
Patient antiserum is added; complementary antibody binds to antigen
3
Enzyme-linked anti-HG is added and binds to bound antibody
4
Enzyme's substrate (□) is added, and reaction produces a visible color change (●)
B

**Figs 6.23A and B:** Enzyme-linked immunosorbent assay (ELISA). **A.** A positive direct ELISA to detect antigens; **B.** A positive indirect ELISA to detect antibody.

will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in direct ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample the lower the absorbance (Fig. 6.24).

Many ELISA tests are available for clinical use in the form of commercially prepared kits. Procedures are often highly automated with the results printed out directly by computer. Simple diagnostic tests including the card and dipstick methods are suitable for clinical laboratory and bedside application. Cassette ELISA (tridot) test can yield result within 10 minutes. There is no need for microplate washer or ELISA reader. One can read visually. Inbuilt negative and positive controls are there.

### *Enzyme-linked Immunospot Assay*

Enzyme-linked immunospot assay (ELISPOT), is a modification of ELISA, which allows detection and quantitative determination of the number of the cells in a population that are producing antibodies specific for given antigen or an antigen for which one has specific antibody. For example, individual B cells producing specific antibody or individual T cells secreting particular cytokines may be detected by ELISPOT assay (Figs 6.25A and B).

*For detection of cells producing specific antibody:* The wells (or plates) are coated with antigen (capture antigen) recognized by the antibody of interest. A suspension of lymphocytes (B lymphocytes) under investigation is then added on to the antigen sensitized well. Secreted antibody binds antigen in the immediate vicinity of the cells producing specific antibody. This spots of bound antibody are then detected chromatographically using enzyme coupled to anti-immunoglobulin and a chromogen.

*For detection of cytokine-producing cells:* The wells (or plates) are coated with antibody against the antigen of interest, a cytokine in this example. A suspension of lymphocytes, under investigation, is then added to the well. Most of the cytokine molecules secreted by a particular cell react with near by well-bound anticytokine antibodies. The captured cytokine is detected by an enzyme and chromogen coupled with anticytokine antibody produced in goat or rabbit. The colored products precipitate and form a spot only on the areas of the well where cytokine-producing cells are deposited. By counting the number of colored spots, it is possible to determine the number of cytokine-producing cells.

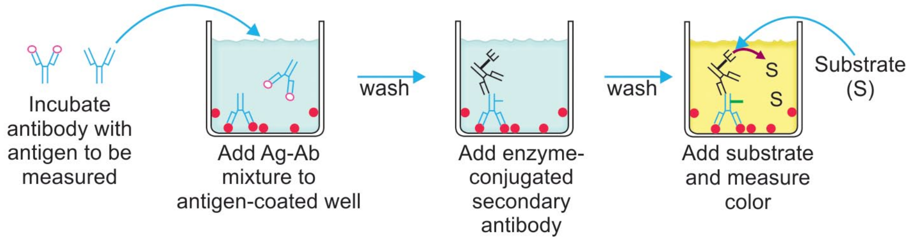
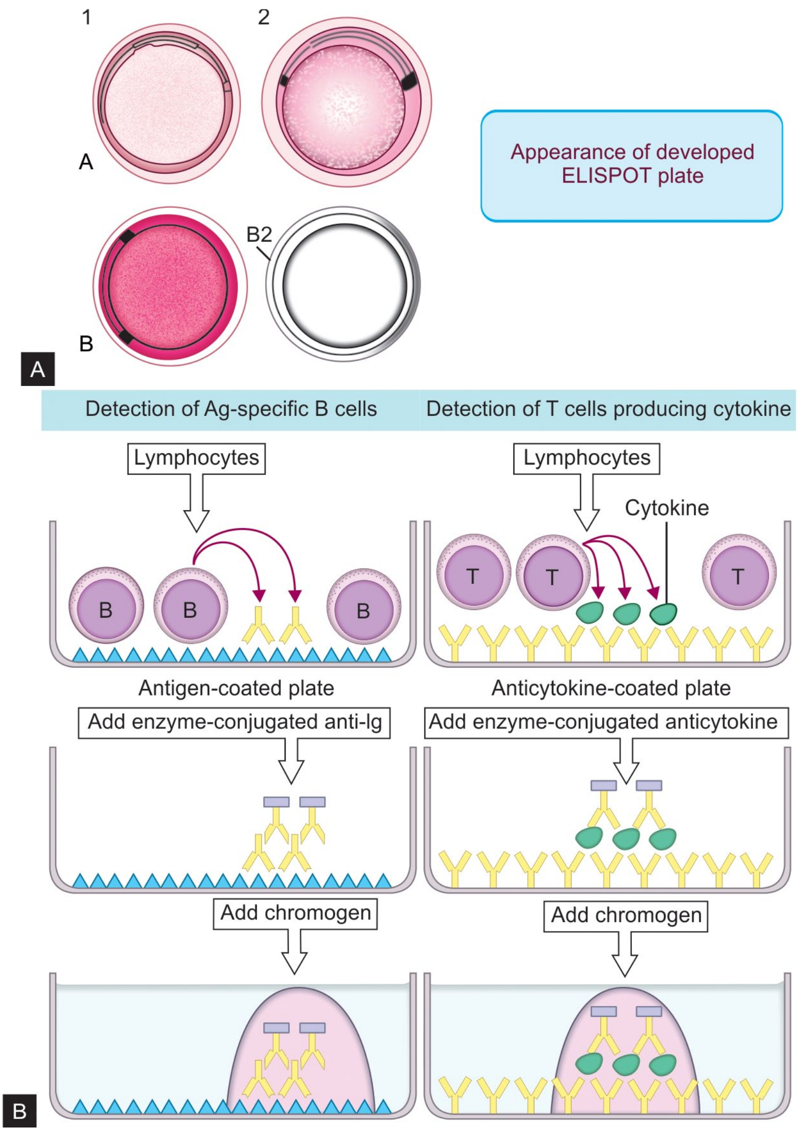

Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
wash
Add enzyme-conjugated secondary antibody
wash
S
S
Substrate (S)
Add substrate and measure color

**Fig. 6.24:** Competitive enzyme-linked immunosorbent assay (ELISA). It is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.


1
2
A
B2
B
Appearance of developed ELISPOT plate
A
Detection of Ag-specific B cells
Detection of T cells producing cytokine
Lymphocytes
Lymphocytes
Cytokine
B
B
B
T
T
T
Antigen-coated plate
Anticytokine-coated plate
Add enzyme-conjugated anti-Ig
Add enzyme-conjugated anticytokine
Add chromogen
Add chromogen
B

**Figs 6.25A and B**: Enzyme-linked immunospot (ELISPOT) assay. **A.** From photographic courtesy of R Hutchings and Blackwell Scientific; **B.** Schematic representation.

*Biotin/Avidin Enhanced Immunoassay*

In many situations, the sensitivity of these assays can be further enhanced by using additional steps in the reaction. Most commonly this is done by the use of secondary antibodies labeled with vitamin biotin; wells are then incubated with enzymatically labeled avidin (a protein component of egg white), which binds biotin with extreme high affinity and specificity (Figs 6.26A and B). Biotin/avidin enhanced immunoassays allows for antigen detection using extremely small sample. Biotin/avidin enhanced assay are also used in immunofluorescence assays (IFA).

Immunofluorescence assays can also take the advantage of biotin-avidin amplification method used in ELISA assays. In this case, the primary antibody is labeled with biotin and fluorescent-labeled avidin is used for detection. Because of the high affinity and specificity of the biotin-avidin interaction, this method significantly improves the sensitivity of IFA.

## Chemiluminescence

Measurement of light produced by chemiluminescence during a certain chemical reaction provides a convenient and highly sensitive alternative to absorbance measurements in ELISA. In versions of the ELISA using chemiluminescence, a luxogenic (light generating) substrate takes the place of chromogenic substrate in conventional ELISA reaction.

## Immune Blotting

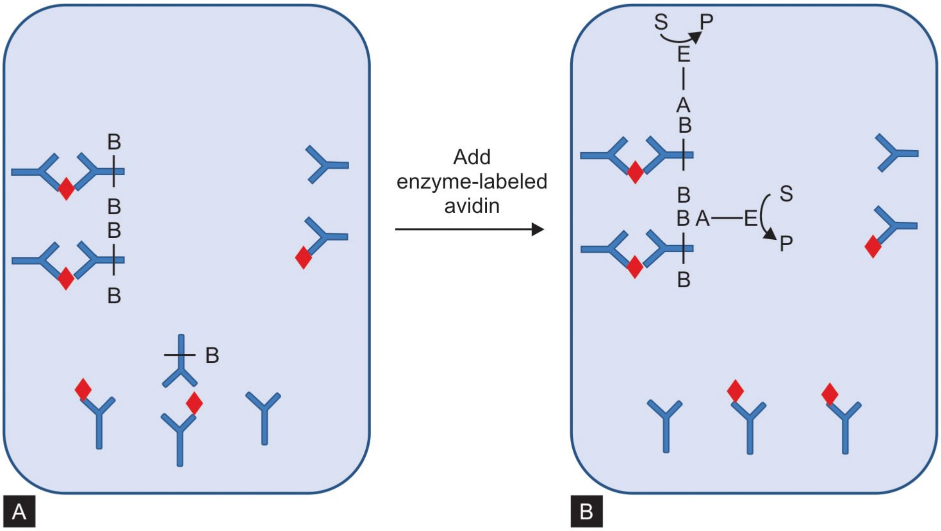
### *Western Blot Test*

Western blotting technique is used for identification of a specific protein in a complex mixture of proteins.

The commonest example is the isolation of human immunodeficiency virus (HIV) proteins in individual suffering from HIV infection. In western blotting, HIV proteins are isolated from the individual and are first separated in a gel by an electric current similar to the procedure used in immunoelectrophoresis. The separated proteins are then transferred (blotted) to cellulose filter paper. Next, serum from the individual is added to the blot. If the HIV antibodies are present, they will react with separated HIV proteins. Such antigen-antibody complexes can be visualized by the addition of an enzyme-labeled antihuman antibody. When an enzyme substrate is added, colored bands appear on the paper. Thus, western blotting can determine the exact viral antigens to which the HIV antibodies are specific (Figs 6.27A to C).

## Flow Cytometry and Fluorescence

The fluorescent antibody techniques are extremely valuable qualitative tools, but they do not provide quantitative data. This short



**Figs 6.26A and B**: Example of biotin/avidin-enhanced ELISA sandwich assay. **A.** Monoclonal antibody (MAb) coated on the wall of the microtiter well, reacts with test antigen. A specific antibody labeled with biotin (B) added to form a sandwich; **B.** Enzyme-labeled avidin (A) is added. The binding of avidine is monitored by the conversion of the substrate (S) to a colored product (P).

coming was overcome by development of flow cytometry a version, which is called fluorescent activated cell sorter (FACS), which was designed to automate the analysis and separation of cells stained with fluorescent antibody. The FACS uses a laser beam and light detector to count single intact cells in suspension (Fig. 6.28).

### Immunoelectron Microscopic Test

#### *Immunoelectron Microscopy*

When viral particles mixed with specific antisera are observed under the electron microscope they are seen to be clumped. This method is used in the study of some viruses such as hepatitis virus A and other viruses causing diarrhea.

#### *Immunoferritin Test*

Ferritin can be conjugated with antibody and such labeled antibodies with antigen can be visualized under electron microscope.

#### *Immunoenzyme Test*

Some enzymes such as peroxidase can be conjugated with antibodies. Tissue sections with corresponding antigens are treated with peroxidase-labeled antisera. The peroxidase bound to the antigen can be visualized.

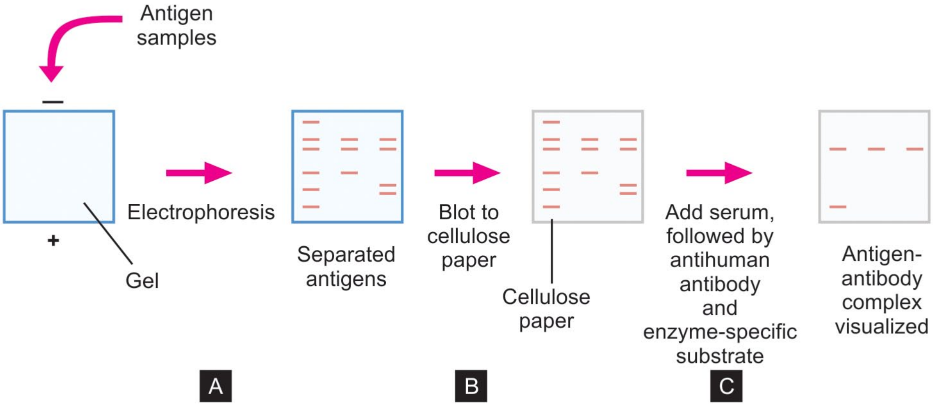
## STUDY QUESTIONS

### Essay Questions

1. Enumerate antigen-antibody reactions. Explain the effects of excess of antigen and antibody on precipitation reaction.
2. Mention antigen-antibody reactions. Discuss the agglutination reactions with their applications.
3. Explain fluorescent antibody techniques.
4. Explain ELISA techniques.
5. Explain the principle of complement fixation test.

### Short Notes

1. Zone phenomenon.
2. Gel diffusion.
3. Opsonization.
4. Passive agglutination.
5. Coagglutination.
6. Neutralization.
7. Western blot test.


Antigen samples
–
+
Electrophoresis
Gel
Separated antigens
Blot to cellulose paper
Cellulose paper
Add serum, followed by antihuman antibody and enzyme-specific substrate
Antigen-antibody complex visualized
A
B
C

**Figs 6.27A to C:** Western blotting test for human immunodeficiency virus (HIV) antigens in blood. **A.** HIV antigens in a gel are separated by an electric current, forming bands of separate antigens; **B.** The antigen bands are transferred (blotted) to cellulose paper; **C.** HIV antibodies tagged with dye are added. Any antibodies recognizing specific HIV antigen attach to that antigen and form a visible band.

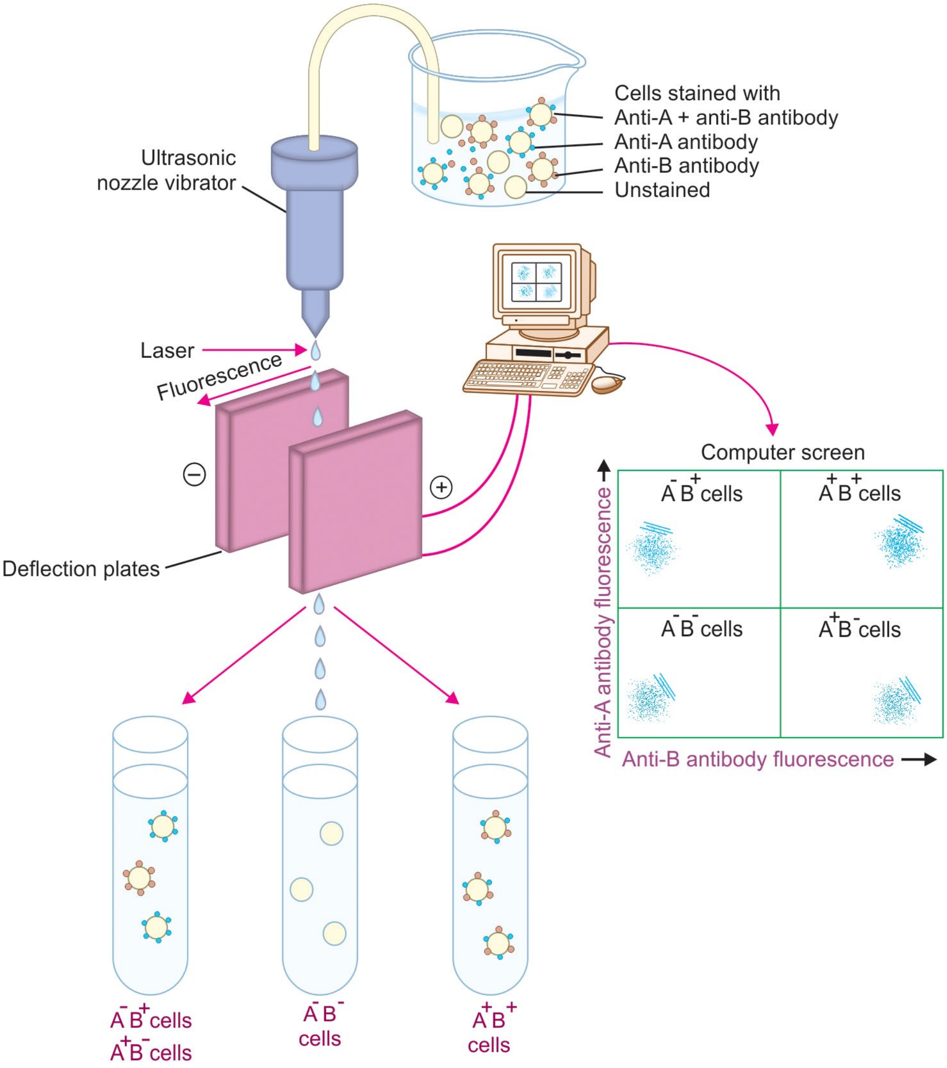

Cells stained with
Anti-A + anti-B antibody
Anti-A antibody
Anti-B antibody
Unstained
Ultrasonic nozzle vibrator
Laser
Fluorescence
⊖
⊕
Deflection plates
Computer screen
$A^-B^+$ cells
$A^+B^+$ cells
$A^-B^-$ cells
$A^+B^-$ cells
Anti-A antibody fluorescence
Anti-B antibody fluorescence
$A^-B^+$ cells
$A^+B^-$ cells
$A^-B^-$ cells
$A^+B^+$ cells

**Fig. 6.28:** Separation of fluorochrome-labeled cells with the fluorescence-activated cell sorter (FACS). In the example shown, a mixed cell population is stained with two antibodies, one specific for surface antigen A and the other specific for surface antigen B. The anti-A antibodies are labeled with fluorescein (blue) and the anti-B antibodies with rhodamine (brown). The stained cells are loaded into the sample chamber of the FACS. The cells are expelled, one at a time, from a small vibrating nozzle that generates microdroplets, each containing not more than a single cell. As it leaves the nozzle, each droplet receives a small electrical charge and the computer that controls the flow cytometer can detect exactly, when a drop generated by the nozzle passes through the beam of laser light that excites the fluorochrome. The intensity of the fluorescence emitted by each droplet that contains a cell is monitored by a detector and displayed on a computer screen. Because the computer tracks the position of cache droplet, it is possible to determine when a particular droplet will arrive between the deflection plates. By applying a momentary charge to the deflection plates when a droplet is passing between them, it is possible to deflect the path of a particular droplet into one or another collecting vessel. This allows the sorting of a population of cells into subpopulations having different profiles of surface markers.

## SUGGESTED READING

1. Black JG. Microbiology: Principles and Applications, 3rd edition. USA: Prentice Hall College div; 1996.
2. Colligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
3. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
4. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
5. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
6. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
7. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby: Elsevier; 2006.
8. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's Illustrated Reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
9. Tortora, Funke, Case. Microbiology an Introduction, 6th edition. 1998.

# Complement System

# 7

The complement system includes serum and membrane-bound proteins that functions in both natural and acquired defense system. These proteins are highly regulated and interact via a series of proteolytic cascades. The term 'complement' refers to the ability of these proteins, to complement (augment) the effects of other components of the immune system (e.g. antibody). Complement has several main effects:

1. Lysis of cells (e.g. bacteria, tumor cells and enveloped virus directly).
2. Production of peptide fragments that participate in inflammation and attract phagocytes.
3. Opsonization of organisms and immune complexes for clearance by phagocytosis and enhancement of antibody-mediated immune responses. Further more the complement goes to work, as soon as an invading microbe is detected; the system makes up an effective host immune defense long before specific host defenses are mobilized.

## COMPLEMENT ACTIVATION

The complement system works as a cascade. A cascade is a set of reactions that amplify some effects, i.e. more products are formed in the second reaction than the first, still more in the third and so on. Of the proteins, so far identified in the complement system, 13 participate in the cascade itself, seven activate or inhibit reactions in the cascade. The components of the classical pathway are numbered from C1 to C9 and the reaction sequence is C1-C4-C2-C3-C5-C6-C7-C8-C9. Up to C5, activation involves proteolytic cleavage, liberating smaller fragments from C2 through C5 except for C2, where for historical reasons the larger fragment that remains bound to the complex is termed C2a, the smaller fragments are by the letter 'a' (e.g. C4a) and the larger fragments by letter 'b' (e.g. C5b). Activation of the complement system can be initiated, either by antigen-antibody complexes or by a variety of non-immunological molecules. Sequential activation of complement components occurs via three main pathways.

### Classical Pathway

Only, immunoglobulin M (IgM) and immunoglobulin G (IgG) (IgG1, IgG2, IgG3 not IgG4) activate or fix complement via the classical pathway. The formation of an antigen-antibody complex induces conformational changes in the Fc portion of the IgM molecule that expose a binding site for the C1 component of the complement system. C1 in serum is a macromolecular complex consisting of C1q and two molecules of each of C1r and C1s, held together in a complex ($C1qr_2$, $s_2$) stabilized by $Ca^{2+}$ (Fig. 7.1). One molecule of IgM or two molecules of IgG can initiate the process. C1q binding in the presence

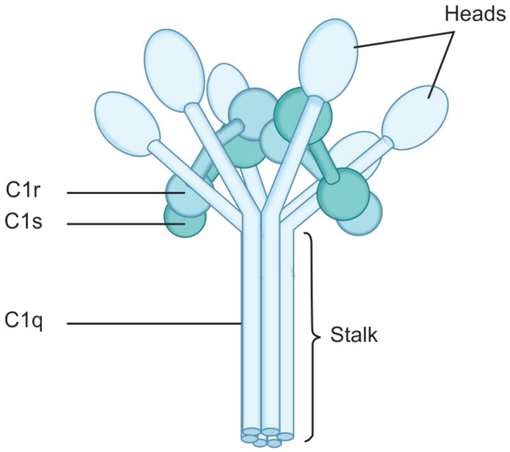


**Fig. 7.1:** C1 macromolecular complex consisting of C1q and two molecules of each C1r and C1s ($1qr_2s_2$)

of calcium ions, leads to sequential activation of C1r and C1s. C1s cleaves C4 and C2 to form C4bC2a, the latter is an active C3 convertase, which cleaves C3 molecule into two fragments as C3a and C3b. C3a is an anaphylatoxin. C3b forms complex with C4bC2a producing a new enzyme called C5 convertase (C4bC2aC3b). C5 convertase cleaves C5 to form C5a and C5b. C5a is an anaphylatoxin and chemotactic factor. The C5b component is extremely labile and becomes inactive within 2 minutes, unless C6 binds to it and stabilizes its activity. Attachment of C5b to the bacterial membrane initiates formation of the membrane attack complex (MAC) and lysis of the cell (Fig. 7.2). The attachment of C5b leads to the addition of components C6, C7 and C8. C8 provides a strong anchor into the membrane and facilitates the subsequent addition of multiple C9 molecules to form a pore in the membrane. Loss of membrane integrity results in the unregulated flow of electrolytes and causes lysis and death of cell.

*Non-immunological classical pathway activators:* Certain bacteria (e.g. some strains of *Escherichia coli, Salmonella* of low virulence), viruses (parainfluenza virus, human immunodeficiency virus) and even apoptotic cells interact with C1q directly, causing C1 activation and there by classical pathway.

## Alternative Pathway (Properdin Pathway)

The alternative pathway does not involve immune complex. Many unrelated substances such as bacterial endotoxin, IgA and IgD antibodies, cobra venom factor, nephritic factor (protein present in the serum of glomerulonephritis) initiate alternative pathway. This pathway does not involve C1, C2 or C4. C3 is cleaved so that C3 convertase is generated via the action of factors B, D and properdin. The alternative C3 convertase (C3bBb) generates more C3b. The additional C3b binds to the C3 convertase to form C3bBbC3b, which is the C5 convertase of the alternative pathway that generates C5b, leading to production of MAC described earlier (Fig. 7.3).

## Mannan-binding Lectin Pathway

Lectins are proteins that bind to specific carbohydrate. Mannose-binding lectin (MBL) or mannan-binding lectin is an acute phase protein, which binds sugar residues like mannose, found on microbial surface *(e.g. Listeria* species, *Salmonella* species, *Candida albicans).* MBL level can rise rapidly in response to infection, inflammation and other forms of stress. MBL once bound to appropriate mannose containing residues, can interact with MBL-activated serum protease (MASP). Activation of MASP leads to subsequent activation of components C2, C4 and C3 (Fig. 7.4).

## Regulation of the Complement System

Following the activation of the complement, its components and split products are capable of attacking host cells, as well as foreign

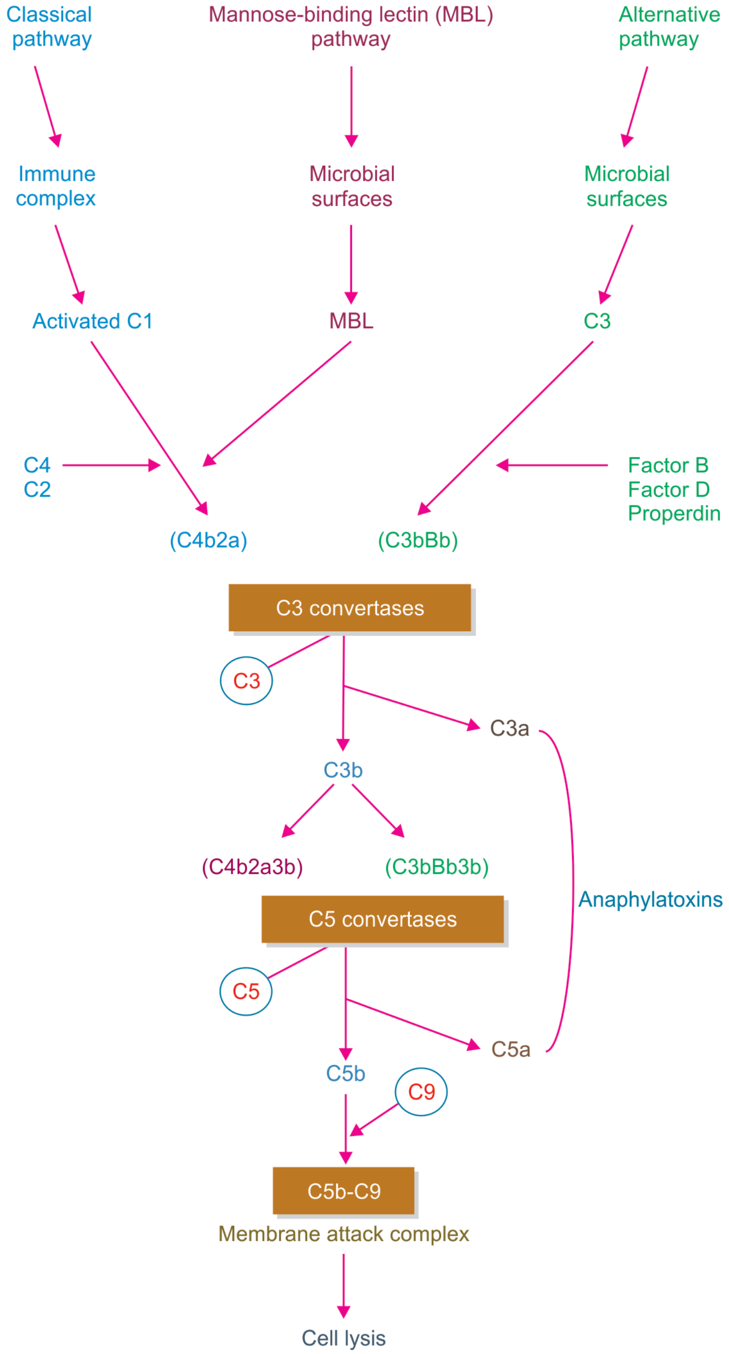

Classical pathway
Mannose-binding lectin (MBL) pathway
Alternative pathway
Immune complex
Microbial surfaces
Microbial surfaces
Activated C1
MBL
C3
C4
C2
Factor B
Factor D
Properdin
(C4b2a)
(C3bBb)
C3 convertases
C3
C3a
C3b
(C4b2a3b)
(C3bBb3b)
Anaphylatoxins
C5 convertases
C5
C5a
C5b
C9
C5b-C9
Membrane attack complex
Cell lysis

**Fig. 7.2:** Complement pathway

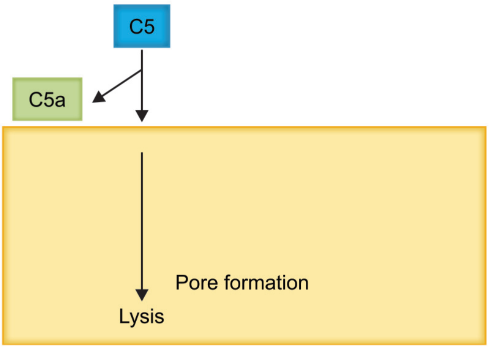


**Fig. 7.3:** Terminal or membrane attack complex (MAC) of complement. The MAC forms pore on the surface of microbes causing lysis.

cells and microorganisms. Elaborate regulatory mechanisms have been evolved, to direct activity on designated targets rather than the host cells. First of all, the components formed in the complement pathway are highly labile substances that stabilized by reaction with other components. Secondly, a series of regulatory proteins can inactivate various complement components disallowing over activation and tissue damage. The activation of complement system is regulated at different stages.

### *Regulation Before Assembly of Convertase Activity*

1. C1 inhibitor (C1 INH) binds to $C1r_2s_2$, causing dissociation from C1q.
2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4b BP), complement receptor type1 (CR1) or membrane cofactor protein (MCP).
3. Inhibitor-bound 4b is cleaved by factor I to form bound 4d and soluble 4c.
4. In alternate pathway, CR1, MCP or factor H prevent binding of C3b and factor B.
5. Inhibitor-bound C3b is cleaved by factor I to form iC3b and soluble C3f fragment. Further cleavage of iC3b by factor I releases C3c and leaves C3dg bound to the membrane.

### *Regulation After Assembly of Convertases*

1. C3 convertases are dissociated by C4b BP, CR1, factor H and decay-accelerating factor (DAF).
2. Factor 'P' (properdin) protects C3b and stabilizes this C3 convertases of the alternative pathway.
3. Anaphylatoxin inactivator is an alpha globulin that enzymatically degrades C3a, C4a and C5a, which are anaphylatoxins released during 'C' cascade.

### *Regulation at Assembly of Membrane Attack Complex*

1. 'S' protein prevents insertion of C5b678 MAC components into the membrane.
2. Homologous restriction factor (HRF) and membrane inhibitor of reactive lysis (MIRL or CD59) binds C81 preventing assembly of poly C9 and blocking formation of MAC.

## Biological Consequences of Complement Activation

Complement plays an important role in humoral immunity by amplifying the response and converting into an effective defense mechanism to destroy invading pathogens. The MAC mediates cell lysis, while the other complement components or split products take part in inflammation, opsonization of antigen, viral neutralization and clearance of immune complexes. Many biological activities of the components are the outcome of the interactions of complement fragments with their receptors expressed on various cells. Some of the complement receptors also help in regulating complement activity by binding, biologically active complement components and degrading them into inactive form. The complement receptors and their primary ligands, which include various complement components and their

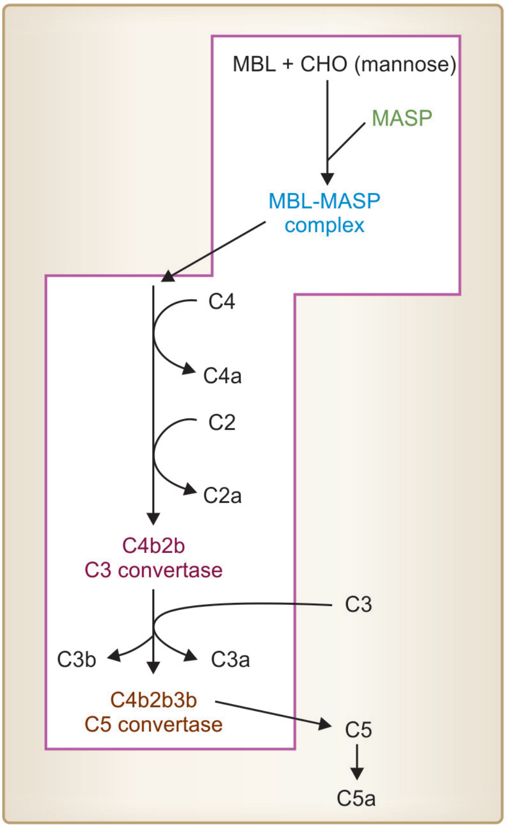


**Fig. 7.4:** Mannose-binding lectin (MBL) pathway of complement activation. MBL bound to mannose containing residues (–CHO) interact with MBL-activated serine proteases (MASP). Activation of MASP leads to activation of component C2, C4 and C3.

proteolytic products are listed (Table 7.1). The major biological effects of the complement (Figs 7.5A and B) are:

1. Opsonization: Cells, immune complexes are easily phagocytosed much more efficiently in the presence of C3b receptors in most of the cells.
2. Chemotaxis: C3a and C5a stimulate the movement of neutrophils.
3. Inflammation (C3a, C4a and C5a).
4. Anaphylatoxins: C3a, C4a and C5a can stimulate the degranulation of mast cells, releasing mediators.
5. Phagocytic function is carried out by C3b and C5b.
6. Increased capillary permeability: C2 kinins are vasoactive amines, which increases capillary permeability.
7. Virus neutralization: May require participation of 'C' for neutralization of herpes virus by IgM antibody.
8. Bacteriolysis or cytolysis: Insertion (C5b 6789, MAC) into the cell surface leads to killing or lysis of erythrocytes, bacteria and tumor cells.
9. Immune adherence: 'C' bound to immune complex adhere to erythrocytes or to non-primate platelets. The immune adherence (C3 and C4) contributes to defense against pathogenic microorganisms, since such adherent particles are rapidly phagocytosed.

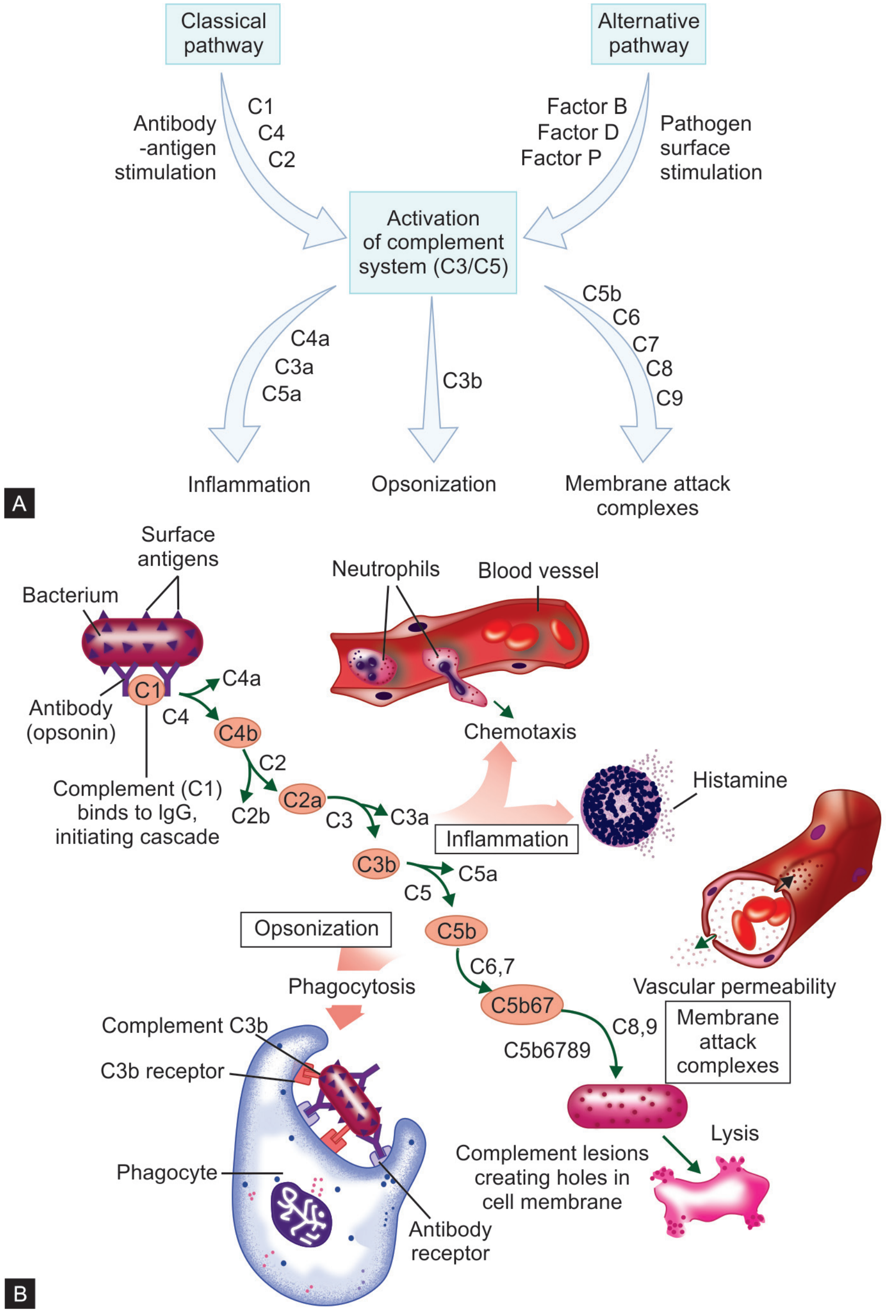
Besides the biological effects stated above, 'C' participate in the cytotoxic (type II) and immune complex (type III) hypersensitivity reactions and play a major role in the pathogenesis of autoimmune hemolytic anemia, paroxysmal nocturnal hemoglobinuria and hereditary angioneurotic edema.

Endotoxin is an effective activator of alternate 'C' pathway. In endotoxic shock, there is massive platelet lysis and release of large amount of platelet factor leads to disseminated intravascular coagulation (DIC) and thrombocytopenia.

## Defects in the Complement System

Deficiency in the complement system affects both innate and acquired immunity. A number of gene defects involving complement components or their regulatory proteins lead to susceptibility to infections or risk to autoimmune disorders.

Homozygous deficiency in any of the earlier components (C1q, C1r, C1s, CH, C2) exhibit similar symptoms, notably marked increase in immune complex disorders, such


Classical pathway
Antibody -antigen stimulation
C1
C4
C2
Alternative pathway
Factor B
Factor D
Factor P
Pathogen surface stimulation
Activation of complement system (C3/C5)
C4a
C3a
C5a
C3b
C5b
C6
C7
C8
C9
Inflammation
Opsonization
Membrane attack complexes
A
Surface antigens
Bacterium
Antibody (opsonin)
C1
C4a
C4
C4b
C2
C2b
C2a
Complement (C1) binds to IgG, initiating cascade
C3
C3a
C3b
C5a
C5
C5b
C6,7
C5b67
C8,9
C5b6789
Neutrophils
Blood vessel
Chemotaxis
Histamine
Inflammation
Vascular permeability
Membrane attack complexes
Opsonization
Phagocytosis
Complement C3b
C3b receptor
Phagocyte
Antibody receptor
Complement lesions creating holes in cell membrane
Lysis
B

**Figs 7.5A and B**: Major biological effects of the complement. **A.** Classical and alternative pathways of the complement cascade. Although the two pathways are initiated in different ways, they combine to activate the complement system; **B.** Activation of the classical complement pathway. In this cascade, each complement protein activates the next one in the pathway. The action of C3b is critical for opsonization and along with C5b for formation of membrane attack complexes. C4a, C3a and C5a also are important to inflammation and phagocyte chemotaxis.

**Table 7.1** Complement binding receptors

| Receptor | Major ligands | Activity | Cellular distribution |
|---|---|---|---|
| CR1 (CD35) | C3b, C4b | Blocks formation of C3 convertase, binds immune complex to cell | B cells, T cells, macrophages, monocytes, neutrophils, erythrocytes, etc. |
| CR2 (CD21) | C3d, C3dg, iC3b | Part of B cell coreceptor, binds Epstein-Barr virus | B cells, some T cells and follicular dendritic cells |
| CR3 (CD11b/18)<br>CR4 (CD11c/18) | iC3b | Bind immune complexes enhancing their phagocytosis, binds cell adhesion molecules on neutrophils | Monocytes, macrophages, neutrophils, natural killer (NK) cells, etc. |
| C3a/C4a receptor | C3a, C4a | Initiate degranulation of mast cells and basophils | Mast cells, basophils, granulocytes |
| C5a receptor | C5a | Induces degranulation of mast cells and basophils | Mast cells, basophils, monocytes, macrophages, platelets, etc. |

as systemic lupus erythematosus (SLE), glomerulonephritis and vasculitis. In addition, the individuals with such complement deficiency are more prone to suffer from repeated pyogenic infections by bacteria such as staphylococci and streptococci.

Deficiency of factor D and properdin leads to increased susceptibility to infection, specially by *Neisseria,* because of impaired alternative pathway.

C3 deficiency results in serious problems with recurrent infections and with immune complex-mediated diseases because of the central position of C3 in all three of the complement activation pathways.

Deficiency in the complement components (C5, C6, C7, C8 and C9) involved in MAC, develop recurrent meningococcal and gonococcal infections. Individuals, deficient of MAC components, rarely suffer from immune complex diseases. This suggests that they produce enough C3b to clear immune complex by opsonization.

Deficiency in regulatory proteins causes serious disorders. The most common is hereditary angioneurotic edema with reduced level of C1 inactivation of classical pathway. As a result uncontrolled inflammatory episodes occur involving vascular system, gastrointestinal (GI) tract and respiratory tract.

Deficiency in DAF or CD59 allow accumulation of complement complexes including the MAC on host cell membrane causing cell injury.

## Synthesis of Complement

Complements are synthesized by liver, spleen and phagocytic cells.

## STUDY QUESTIONS

### Essay Questions

1. What is complement? Explain in detail about complement pathway.
2. Discuss the complement-mediated serological tests.
3. Discuss the biological activities of complements.

### Short Notes

1. Complement.
2. Complement deficiency diseases.
3. Complement receptors.

4. Mannan-binding lectin pathway of complement activation.

## SUGGESTED READING

1. Black JG. Microbiology. Principles and Applications, 3rd edition. USA: Prentice Hall College div; 1996.
2. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current protocols in immunology. New York: Wiley; 1997.
3. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
4. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
5. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
6. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill Lange Basic Science; 2010.
7. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby: Elsevier; 2006.
8. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
9. Tortora, Funke, Case. Microbiology an Introduction, 6th edition; 1998.

# Cells and Tissues of the Immune System

# 8

The cells, which take part in immune reactions are organized into tissue and organs in order to perform their functions most effectively. These structures together are referred to as the lymphoid system. The lymphoid system consists of lymphocytes, macrophages [antigen-presenting cells (APCs)] and epithelial cells in some tissues. These cells are arranged into either discretely capsulated organs or accumulated diffusely in some tissues.

## LYMPHOID ORGANS

The lymphoid organs are classified into two types. They are primary (central) or secondary (peripheral).

### Primary Lymphoid Organs

Primary lymphoid organs are major sites for lymphopoiesis. Here the lymphocytes develop, differentiate from lymphoid stem cells, proliferate and mature into functional cells. In mammals, T cells mature in thymus and B cells in fetal liver and bone marrow (bursa of Fabricius is the site for B cell development) in birds. It is in the primary lymphoid organs the lymphocytes acquire the antigen receptors to fight with the antigenic challenge subsequently during life.

*Thymus (Sites for T Cell Development)*

The thymus of mammals is composed of two lobes, each comprising multiple lobules being separated by connective tissue trabeculae. Lymphocytes (thymocytes) are placed more densely towards the periphery of each lobule than near its center, which gives rise to the outer cortex and inner medulla. The tightly packed outer cortex contains relatively more immature proliferating thymocytes. The medulla contains more mature cells. Mature thymocytes in the medulla express CD44, which is not detected in cortical thymocytes. There is a network of epithelial cells through out the lobules, which plays a role in differentiation processes from stem cells to mature T lymphocytes. The epithelial component is made up of sheets and islands of squamous cells that make and secrete factors that attract T cell precursors from the blood and also promote subsequent maturation within the thymus.

These factors include a chemokine called thymus expressed cytokine (TECK) and a number of small, incompletely characterized peptide hormones known as thymulin, thymopoietin, thymic humoral factors and thymosin. In addition interdigitating dendritic cells (IDCs) and macrophages, both derived from bone marrow, are found particularly in corticomedullary junction. Epithelial cells, IDCs and macrophages express major histocompatibility complex (MHC) molecules, which are crucial to T cell development and selection.

The mammalian thymus involutes with age. In man atrophy starts at puberty and continues through the rest of life. The involution begins with the cortex and the entire cortical areas disappear though medullary remnant persists. It is said that T cell generation in the thymus continues into adult life though at a lower rate.

### *Adult Bone Marrow and Fetal Liver (Sites for B Cell Development)*

In birds, B cells differentiate in bursa of Fabricius, hence the term B cells. Mammals have no bursa; instead, islands of hemopoietic cells in fetal liver and in fetal and adult bone marrow give rise to B cells.

## Secondary Lymphoid Organs

Secondary lymphoid organs are spleen, lymph node and other mucosa-associated lymphoid tissue (MALT), which include tonsils and Peyer's patches. In the secondary lymphoid tissue, the lymphocytes interact with lymphocytes accessory cells (macrophages both phagocytic and antigen-presenting) and also with antigen. The immune response is generated and disseminated in the secondary lymphoid tissues.

The secondary (peripheral) lymphoid tissues consist of well-organized encapsulated organs—the spleen and the lymph nodes and non-encapsulated accumulations that are found throughout the body, specially with the mucosal surfaces and is called MALT. After acquiring immunological competence, in the primary lymphoid organs (thymus and bone marrow) the lymphocytes migrate along the blood and lymph stream, accumulated in their respective sites in lymph nodes and spleen. Following antigenic stimulus they cause the appropriate immune response. The spleen is responsive to blood born antigens and the lymph nodes protect the body from the antigens coming from the skin or internal surfaces via lymphatics.

In contrast, the mucosal system protects from antigens entering the body directly through mucosal epithelial surfaces—lining of the intestinal tract [gut-associated lymphoid tissue (GALT)], the respiratory tract [bronchus-associated lymphoid tissue (BALT)] and the genitourinary tract. The prime effector mechanism is secretory immunoglobulin A (sIgA) secreted directly on to the mucosal surfaces of the tract.

### *Lymph Node*

Human lymph nodes (Fig. 8.1) are 2 to 10 µm in diameter, are round or kidney-shaped. Lymph node has an indentation called hilus through which blood vessels enter and leave. Lymph arrives at the lymph node via several afferent lymphatics and leaves the node through efferent lymphatic vessels at the hilus. Lymph node is surrounded by a fibrous capsule from which trabeculae penetrate into the node. The lymph node consists of a B cell area (cortex), a T cell area (paracortex) and a central medulla, which has cellular cords that has T cells, B cells, plasma cells and abundant macrophages.

The paracortex contains many APCs, which expresses high levels of MHC class II surface antigens. The accumulation of lymphocytes in the cortical area is known as primary follicle. Following antigenic stimulation, germinal center appears, which is known as secondary follicle. Lymph nodes act as a filter from the lymph, each group of nodes draining a specific part of the body. The macrophage phagocytose foreign materials including microorganisms. They help in proliferation and circulation of T and B cells. The lymph nodes enlarge following antigenic stimulation.

### *Spleen*

The spleen (Fig. 8.2) filters blood much as the lymph nodes filter lymph. It is enclosed in a thin and rather fragile connective

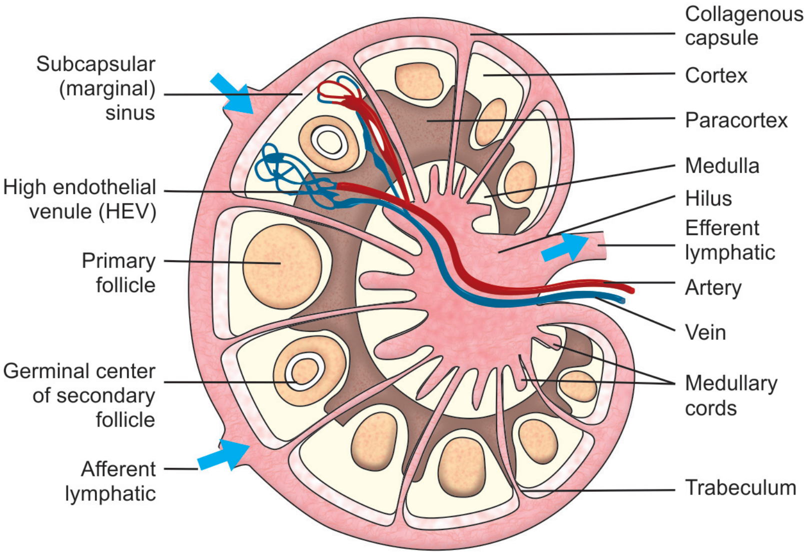


**Fig. 8.1:** Schematic structure of the lymph node. Beneath the collagenous capsule is the subcapsular sinus, which is lined with phagocytic cells. Lymphocytes and antigens from surrounding tissue spaces or adjacent nodes, pass into the sinus via the afferent lymphatics. The cortex contains aggregates of B cells (primary follicles) most of which are stimulated (secondary follicles) and have a site of active proliferation or germinal center. The paracortex contains mainly T cells, many of which are associated with the interdigitating cells (antigen-presenting cells). Each lymph node has its own arterial and venous supply. Lymphocytes enter the node from the circulation through the specialized high endothelial venules (HEVs) in the paracortex. The medulla contains both T and B cells, as well as most of the lymph node plasma cells organized into cords of lymphoid tissue. Lymphocytes can only leave the node through the efferent lymphatic vessel.

tissue capsule, which penetrate into the parenchyma of the organ as trabeculae. The branches of the splenic artery (trabecular artery) travel along the trabeculae and on leaving them branch again, to form the central arterioles. Each arteriole is encased in a cylindrical cuff of lymphoid tissue that mainly consists of mature T cells and are called periarteriolar lymphoid sheath (PALS). Primary and secondary lymphoid follicles protrude at intervals from the sheath. These are identical to the follicles found in other lymphoid tissues and are composed mainly of B cells surrounding the sheath and lymphatic follicles. Together the region is called marginal zone, composed mainly of B cells and macrophages.

The arterioles, sheaths, follicles, marginal zones and a small amount of associated connective tissue are together called white pulp.

The lymphatic sheath immediately surrounding the central arteriole is thymus dependent area of the spleen. The perifollicular region, germinal center and mantle layer form the B cell-dependent areas.

Blood flows from the arterioles into the red pulp, a spongy blood filled network of reticular cells and macrophage lined vascular sinusoids that makes of the bulk, of the spleen and then exits by way of the splenic veins.

During the course of each day, approximately half the blood volume passes through the spleen where lymphocytes, dendritic cells and macrophages survey for evidence of infectious agents. Then the spleen serves as a critical line of defense against blood-borne pathogens. Spleen, besides acting as a blood filter, also serves eliminating abnormal damaged and senescent red or white cells from the blood.

## Tonsils, Peyer's Patches and other Subepithelial Lymphoid Organs

Dense population of T and B lymphocytes, plasma cells macrophages can normally be found below the mucosal lining of alimentary system, genitourinary system and the respiratory system, to detect any foreign substances that contact these body surfaces. In most areas, the cells form diffuse disorganized mass with occasional isolated lymphoid follicle.

At other site, the cells are organized into discrete stable anatomic structures such as tonsils, Peyer's patches. Tonsils are nodular aggregates of macrophages and lymphoid cells, without a capsule, lies beneath the

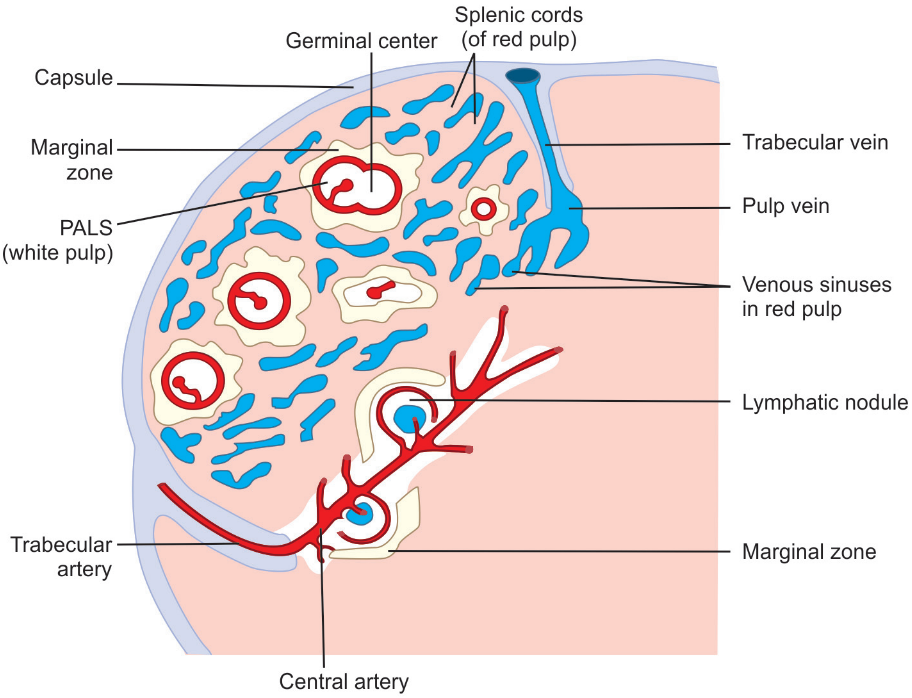

Splenic cords
(of red pulp)
Germinal center
Capsule
Marginal zone
PALS
(white pulp)
Trabecular vein
Pulp vein
Venous sinuses
in red pulp
Lymphatic nodule
Marginal zone
Trabecular artery
Central artery

**Fig. 8.2**: Simplified diagram of segment of spleen. The white pulp is composed of periarteriolar lymphoid sheaths (PALS), frequently containing germinal centers with mantle zones. The white pulp is surrounded by the marginal zone, which contains numerous macrophages, APCs, slowly recirculating B cells and NK cells. The red pulp contains venous sinuses separated by splenic cords. Blood enters the tissues via the trabecular arteries, which give rise to the many-branched central arteries. Some end in the white pulp, supplying the germinal centers and mantle zones, but most empty into or near the marginal zones. Some arterial branches run directly into the red pulp, mainly terminating in the cords. The venous drain blood into the pulp veins and then into the trabecular veins.

stratified squamous epithelium of the nasopharynx and soft palate. Tonsils detect and respond to pathogens in the respiratory and alimentary tract. Similar to tonsils, some uncapsulated lymphoid nodules are present in the iliac submucosa of small intestine, called Peyer's patches. They serve to detect the substances that diffuse across the epithelial surfaces. Together all of the organized and diffuse lymphoid tissues are known as MALT. Such lymphoid tissue in the gut and bronchial mucous membrane are known as GALT and BALT respectively. One important function of these tissues is to secrete antibodies across the mucosal surface, as a defense against external pathogens.

Skin too is an important site of immunosurveillance. Small populations of lymphocytes are constantly present in the dermis and epidermis, although they do not form lymphatic nodules.

## CELLS OF THE LYMPHORETICULAR SYSTEM

1. Cells involved in immunological functions are as follows:
    a. Lymphocytes.
    b. Plasma cells.
    c. Phagocytic cells.
        - Macrophages
        - Neutrophils
        - Eosinophils
        - Dendritic cells.
2. Structural cells.
    a. Reticulum cells.
    b. Endothelial cells.
    c. Fibroblasts.

### Lymphocytes

The typical lymphocyte is a small round or club-shaped cell, 5 to 12 µm in diameter with a spherical nucleus, densely compacted nuclear chromatin with a thin rim of cytoplasm. Despite their uniform appearance, several different types of lymphocytes can be distinguished on the basis of their functional properties and by specific surface markers they express.

The most fundamental distinction is the division of these cells into two major lineages known as T (thymus derived) cells and B (bone marrow derived) cells. The relative proportions of T and B cells vary in tissue to tissue, but in peripheral blood they constitute 75% and 15% respectively. The remaining 10 percent are a special class of granular lymphocytes known as natural killer (NK) cells.

'T' and 'B' lineage cells, both arise from a subset of hemopoietic stem cells in the bone marrow or fetal liver that become committed to the lymphoid path of development (Fig. 8.3). There are evidences that both T and B cells, as well as NK cells are the descendants of a common committed marrow progenitor cell called lymphoid stem cells. The growth of early lymphoid progenitors require at least two cytokines, called interleukin-7 (IL-7) and stem cell factor (SCF), found in both the thymic and marrow microenvironments. The progeny of these putative stem cells follow divergent pathways to mature into either T or B cell. Human B lymphocytes develop exclusively in bone marrow. T cells, on the other hand, develop from the mature precursor that leave the marrow and travel through the bloodstream to the thymus, where they proliferate and differentiate into mature T lymphocytes under the influence of a number of thymic hormones.

Mature lymphocytes that come out of thymus and bone marrow are in a resting state (mitotically inactive). Dispersed into the bloodstream these, so called naive (virgin), lymphocytes migrate efficiently into various secondary lymphoid organs such as spleen, lymph nodes, tonsils, etc. The function of the secondary organs is to maximize encounters

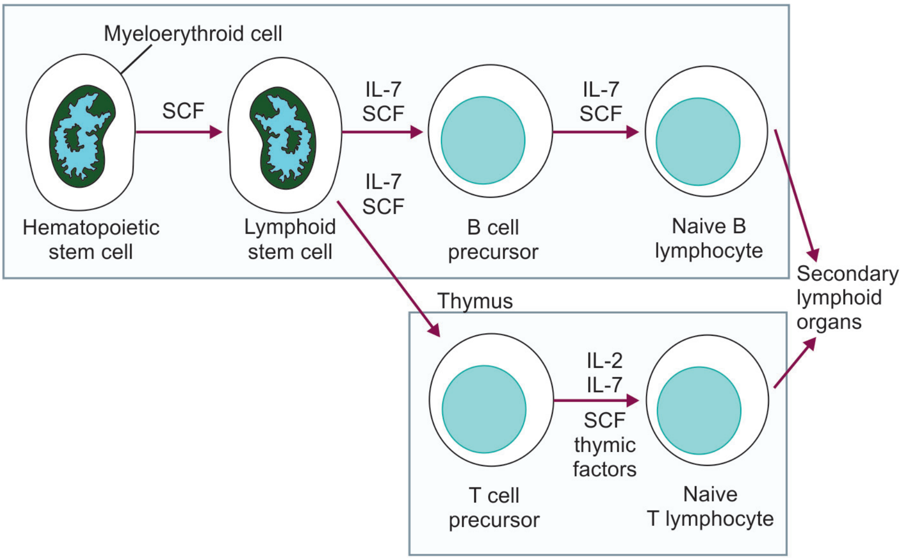

Myeloerythroid cell
SCF
IL-7
SCF
IL-7
SCF
IL-7
SCF
Hematopoietic stem cell
Lymphoid stem cell
B cell precursor
Naive B lymphocyte
Secondary lymphoid organs
Thymus
IL-2
IL-7
SCF
thymic factors
T cell precursor
Naive T lymphocyte

**Fig. 8.3**: Schematic overview of lymphocyte development (lymphopoiesis). In this simplified diagram, most intermediated stages are omitted. The characteristics of cells that migrate to the thymus are unknown. A few of the regulatory molecules needed for proliferation at particular stages of development are indicated (SCF, stem cell factor; IL, interleukin).

between lymphocytes and foreign substances. And, it is from these sites most immune responses are carried out.

Most virgin lymphocytes have an inherently short lifespan and are programmed to die, within few days after leaving the marrow or thymus. However, if such a cell receives signals that indicate the presence of foreign substance or pathogen, it may respond through a phenomenon known as activation. Such, activated or committed or sensitized cell undergoes successive cell division to form memory lymphocytes (Fig. 8.4), which can survive for years and the effector lymphocytes, which survive only for few days, but carry out specific defense activities against the foreign invader.

### *B Cells*

B lymphocyte precursors, pro-B cells, develop in the fetal liver during embryonic life and in the bone marrow afterwards. The important feature of the cell, is its ability to synthesize proteins called immunoglobulin (Ig). Each Ig molecule binds specifically and with high affinity with its own molecular ligand known as antigen.

A virgin (resting) B cell is one-stage of B cell, which has not had contact with antigen. Each resting lymphocyte may express good number of membrane Ig on its surface. On contact with its appropriate antigen, the mature B cell undergoes clonal proliferation. Some activated B cells become long-living memory cells responsible for the recall phenomenon seen in subsequent contact with the same antigen. The majority of the activated B cells are transformed into plasma cells (Fig. 8.5).

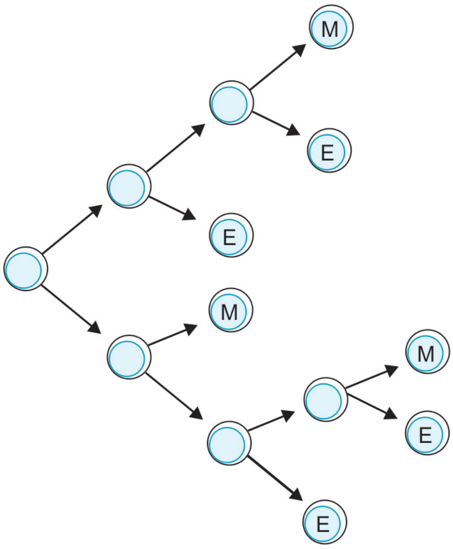


**Fig. 8.4:** Lymphocyte activation. This leads to both cell division and differentiation. At each cell division, individual cells can cease dividing and differentiate into memory (M) or effector (E) cells. In this example, a single activated lymphocyte gives rise to four effector memory cells after four cycles of division.

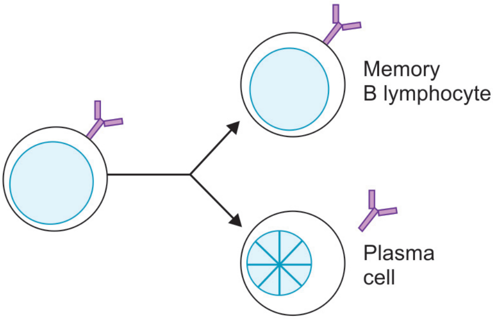


**Fig. 8.5:** The progeny of an activated B lymphocyte can differentiate into either memory B lymphocytes or antibody-secreting plasma cells

### *Surface Markers of B Cell at Different Stages of Development*

Various surface markers identify the developmental B lineage cells such as pro-B and pre-B cells. At the pro-B stage, the cells do not display the heavy or light chains of antibody, on the other hand, they do express CD45R, which is a form of the protein tyrosine phosphatase found on leukocytes and the signal transduction molecules Igα/Igβ, which are found in association with the membrane forms of antibody in later stages of B cell development (Fig. 8.6).

In addition pro-B cells express CD19 (part of B cells coreceptor), CD43 (leukosialin), CD24 [heat stable antigen (HSA)] and C-kit (receptor for a growth promoting ligand present in the stromal cells). While developing from pro-B to pre-B cells, they cease to express some receptors (c-kit and CD43) and begin to express some other receptors (CD25, α-chain of IL-2 receptors and pre-BCR). After rearrangement of light chain, the surface Ig have both heavy and light chains, lose the [B cell receptor (BCR)] pre-BCR and CD25 and then convert to immature B cell.

### *B Cell Subtypes*

There are two subsets of B cells. They are B-1 cells and B-2 cells, which constitute 5% and 95% respectively. B-1 cells are so named, because they are first to develop embryologically that dominate the pleural and peritoneal cavities. They appear during fetal life, express surface IgM, but little or no IgD and are marked by CD5. In contrast the conventional or B-2 cells arise during and after the neonatal period and continuously replaced from the bone marrow and are widely distributed throughout the lymphoid organs and tissues. Each B cell is specific, that is, it produces Ig of one specificity that recognizes only one epitope. The B-1 population of cells responds poorly to protein antigens, but much better to carbohydrates. The antibodies produced by high proportion of B-1 cells are of low affinity.

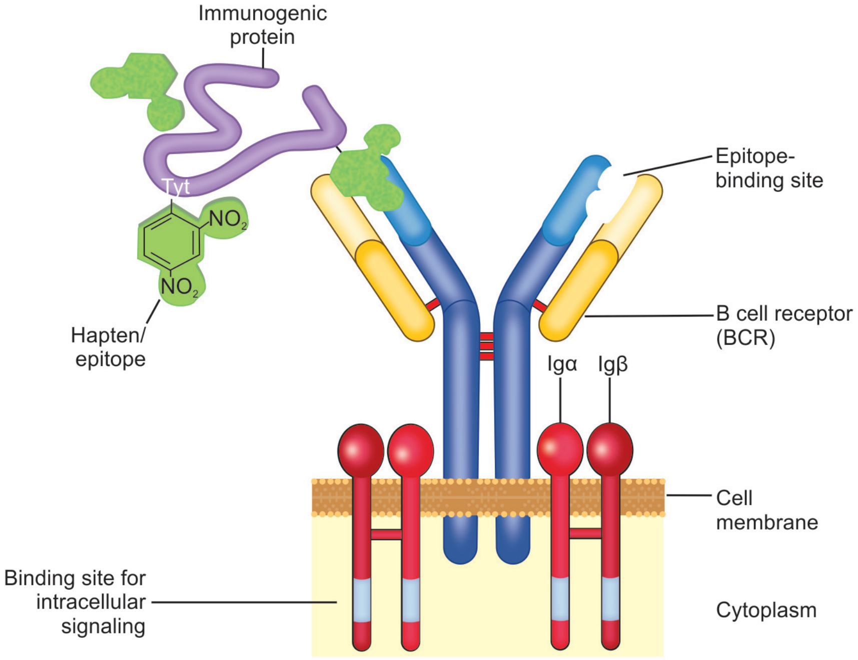


**Fig. 8.6:** Immunoglobulins serve as BCRs. B cells bear receptors that are composed of two identical large (heavy) chains and two identical smaller (light) chains. Molecules such as Igα and Igβ are associated with BCRs and help provide a signal to the cell when the BCR binds an epitope.

## Plasma Cells

Plasma cells are the effector cells of the B lineage, are uniquely specialized to secrete large amount of Ig proteins to the surrounding milieu. Secreted immunoglobulins retain their ability to recognize and bind their specific ligands and are often referred to as antibodies. Binding of the antibodies to their specific ligands have a variety of effects that are beneficial to host cells.

Plasma cells are oval or egg-shaped and have abundant cytoplasm and eccentrically placed round nuclei. Clumps of dark staining chromatin are often distributed around the inner aspect of the nuclear membrane in plasma cell, giving a cart-wheel or clock face appearance under light microscope. The cytoplasm contains abundant rough endoplasmic reticulum and a well-developed Golgi apparatus. Igs are not present in the surface of plasma cell, but produced in large amount in cytoplasm and are then secreted into the extracellular space. Plasma cells have relatively short span of life and are terminally differentiated. The main functions of the 'B' lineage cells are involvement in the following:

1. Humoral acquired immunity.
2. Antigen processing and presenting.
3. Production of an array of cytokines and other factors that influence the growth and activity of other immunologically important cells.

### *T Cells ('T' Lineage Cells)*

A lymphocyte that has been educated by the thymus becomes an immunologically competent cell (ICC). Such cells are qualified to take up immunological response, when approximately stimulated by an antigen. The ICC subserves the function of recognition of antigen, storage of immunological memory and immune response. T lymphocytes do not express Igs, but instead, detect the presence of foreign antigen by way of surface protein called T cell receptors (TCR). These receptors are membrane proteins made up of a pair of polypeptide known as $\alpha$ and $\beta$ chains or $\gamma$ and $\delta$ chains. Large majority of the cells have $\alpha$ and $\beta$ chains. TCR are closely related to immunoglobulins in evolution and share with them a number of structural and functional properties, including the ability to detect specific small molecular ligands called antigens. Unlike Igs however, TCR proteins are never secreted and therefore they are incapable of striking their targets at a long distance. Instead they extend their protective effects, either through direct contact or by influencing the activity of other immune cells. Together with macrophages, T cells are the primary cell type involved in a category of immune response called cell-mediated immunity.

Unlike B cells, T cells can detect foreign substances only in specific context. In particular, T lymphocyte will recognize a foreign protein only if it is first cleaved into small peptides and which are displayed on the surface of a second host cell called antigen-presenting cells (APCs), which constitute macrophages, dendritic cells, B cells and other cell types. Presentation depends in part, on MHC protein on the surface of APC, which is attached non-covalently to the cleaved peptides for display. It is the combination of cleaved peptide and MHC molecule that can attract T cells with appropriate receptors.

### *Surface Molecules (Proteins) on T Lymphocytes*

Mature functional T lymphocytes expresses, a number of characteristic surface proteins in addition to TCR. These molecules have been identified by means of monoclonal antibodies. These markers reflect stage of differentiation and functional properties of the cell. Many of these proteins are referred by the initials of 'clusters of differentiation' (i.e. CD) followed by a unique identifying number like CD1, CD2, CD3, CD4, etc. Over 80 CD markers have been identified. Some of the CD markers with their cell association and other surface molecules are given (Table 8.1).

Almost all the mature T cells found in the secondary lymph nodes and peripheral blood are $CD2^+$, $CD3^+$, that is, both the cells express CD2 and CD3 molecules on their surface. There are distinct subpopulations of cells with very different immunological functions. These subpopulations of cells express their own surface molecules and are referred to as T cell subsets (Table 8.2). The two most important major T cell subsets are ($CD4^+$, $CD8^-$) and ($CD4^-$, $CD8^+$), which constitute 70% and 25% respectively. Most T lymphocytes that express CD8 proteins are cytotoxic, i.e. they are able to kill cells that have foreign macromolecule on their surfaces. CD8 cells have virucidal effect. T lymphocytes that express CD4 protein are not cytotoxic. They are known as helper T cells ($T_h$), which promote proliferation and maturation and immunologic function of other cell types by the help of specific lymphokines. The two minor subsets of T cells are ($CD4^-$, $CD8^-$) and ($CD4^+$, $CD8^+$), which constitute 4% and 1% respectively. The double negative subsets ($CD4^-$, $CD8^-$) of T lymphocytes, instead of $\alpha$ and $\beta$ polypeptide have $\gamma$ and $\delta$ chains of polypeptide. The function of double-positive T cells ($CD4^+$, $CD8^+$) is unknown. Some distinguishing features between T cell, B cell and macrophage are summarized in Table 8.3.

The correlation of $CD4^+$ or $CD8^+$ with helper ($T_h$) or cytotoxic ($T_C$) function is

**Table 8.1** Some important surface molecules on T lymphocytes

| Marker | Major function or significance |
|---|---|
| T cell receptor | Antigen binding |
| CD3 complex | Single transduction from T cell receptor; lineage-specific marker |
| CD2, CD5, CD7 | Lineage-specific markers |
| CD4 | Subset-specific marker (mainly on helper cells); interaction with class II MHC[*] proteins |
| CD8 | Subset-specific marker (mainly on cytotoxic cells); interaction with class I MHC proteins |
| CD28 | Activation-specific marker; receives B7-mediated costimulation from APC[†] |
| CD40 ligand (CD40L) | Activation-specific marker; delivers contact-mediated help to B cells |
| IL[‡]-2 receptor class II MHC proteins transferrin receptor CD25, CD29, CD54, CD69 | Other activation-specific markers |
| IL-1 receptor<br>IL-6 receptor<br>TNF[§]-α receptor | Other cytokine receptors |
| Fc receptors | Immunoglobulin binding |
| LFA[‖]-1, ICAM[¶]-1 | Cell-cell adhesion molecules |

[*] MHC, major histocompatibility complex; [†] APC, antigen-presenting cell; [‡] IL, interleukin; [§] TNF, tumor necrosis factor; [‖] LFA, leukocyte functional antigen; [¶] ICAM, intercellular adhesion molecule.

**Table 8.2** Major T cell subsets found in blood and peripheral tissues

| Surface phenotype | Predominant function | Proportion of total blood T lymphocytes | T cell receptor type |
|---|---|---|---|
| CD4$^+$ CD8$^-$ | Helper | 70% | α/β |
| CD4$^-$ CD8$^+$ | Cytotoxic | 25% | α/β, rarely γ/δ |
| CD4$^-$ CD8$^-$ | Cytotoxic | 4% | γ/δ |
| CD4$^+$ CD8$^+$ | – | 1% | α/β |

strong, but not absolute. A few CD8$^+$ cells have helper activity and also CD4$^+$ cells have cytotoxic activity. Expression of CD4 and CD8 actually correlates most closely with the type of MHC protein that a T cell can recognize.

### *T Cell Maturation*

T cell precursors migrate to the thymus, after being developed in yolk sac, fetal liver and bone marrow, during the embryonic life and following birth. CD7$^+$ pro T cells are the earliest identifiable cells of 'T' lineage, which acquire CD2 on entering the thymus. Then synthesis of CD3 occurs in the cytoplasm and they become pro T cells. TCR synthesis also takes place. TCR occurs as two pairs of glycoprotein chain either αβ or γδ. Pre T cells differentiate into two lineages, expressing either αβ or γδ TCR.

Contact with self-antigens within the thymus leads, to the destruction of immature T cells carrying the corresponding TCR. Thus self-tolerance or elimination of T cells capable of reacting with the self-antigens take place in the thymus. The uncommitted T cells are also converted to committed T cells, against foreign antigens in thymus only.

T cells also develop MHC restriction so that $CD8^+$ cells respond, to foreign antigen presented along with MHC I molecule and $CD4^+$ cells presented with MHC II molecule.

Immature T cells in the thymus exhibit CD7, CD2, CD3, CD1, CD4 and CD8 besides TCR. On functional maturity, they lose CD1 and differentiate into two major subsets and ($CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$) and two minor subsets. Mature $CD4^+$ $CD8^-$ cell serve helper/inducer function and $CD4^-CD8^+$ cells serve as suppressor/cytotoxic function. The minor subsets such as $CD4^+$ $CD8^+$ and $CD4^-$ $CD8^-$ also present in circulation (Fig. 8.7).

### *Null Cells*

There are some lymphocytes, which lack the features of T cells and B cells. They are known as null cells (large granular lymphocytes), which constitute 5% to 10% of all lymphocytes. The null cells or large granular lymphocytes, may be NK cells, antibody-dependent cellular cytotoxicity (ADCC) cells and lymphokine-activated killer (LAK cells).

Natural killer cells possess spontaneous cytotoxicity towards various target cells. The ability of NK cells to kill tumor cells has been, a focus of research interest and therapeutic trials, but there is little evidence that NK cells normally protect against the development of tumor, instead the most important role for NK cell appears to be against infection by intracellular agents such as virus, bacteria and parasite. The cytotoxicity is not antibody dependant or MHC restricted like cytotoxic T lymphocytes. For their action they also do not require sensitization by antigenic contact.

Unlike T cells, NK cells do not express cell surface CDR/CD3 complex. They also lack CD4 surface molecule. About half of human NK cells express CD8 molecule. But it is not required for natural killing. Most NK cells express CD16 (a receptor for Fc portion of IgG) and CD56 [a variant of neural cell adhesion molecule, (NCAM)]. These receptors are not required for natural killing. They are only required for identification of NK cells, which are generally $CD16^+$, $CD56^+$, $CD3^-$ where as T cells are $CD3^+$, $CD16^-$, $CD56^-$. NK cells bind to the glycoprotein receptor of the target cells and release several cytolytic factors. One of them is perforin, which resemble complement component C9 that causes transmembrane pores through which cytotoxic factors such as tumor necrosis factor-β (TNF-β), serine esterase, lymphotoxins enter the cell and destroy it by apoptosis (programed cell death). NK cells are augmented by γ-interferon.

In addition to receptors mentioned earlier, recent findings suggest that the NK cells bear another set of receptors called killer activation receptors (KARs) and killer inhibition receptors (KIRs) that allow them to recognize host cells (Fig. 3.4). When the KAR is engaged by binding to its carbohydrate ligands, on target cells, 'the kill signal' to the NK cell is activated, causing destruction to the target cells. However, if the KIR is engaged by binding of ligands on the surface of the target cell, then the NK cell receive 'do not kill signal' and target cell survives.

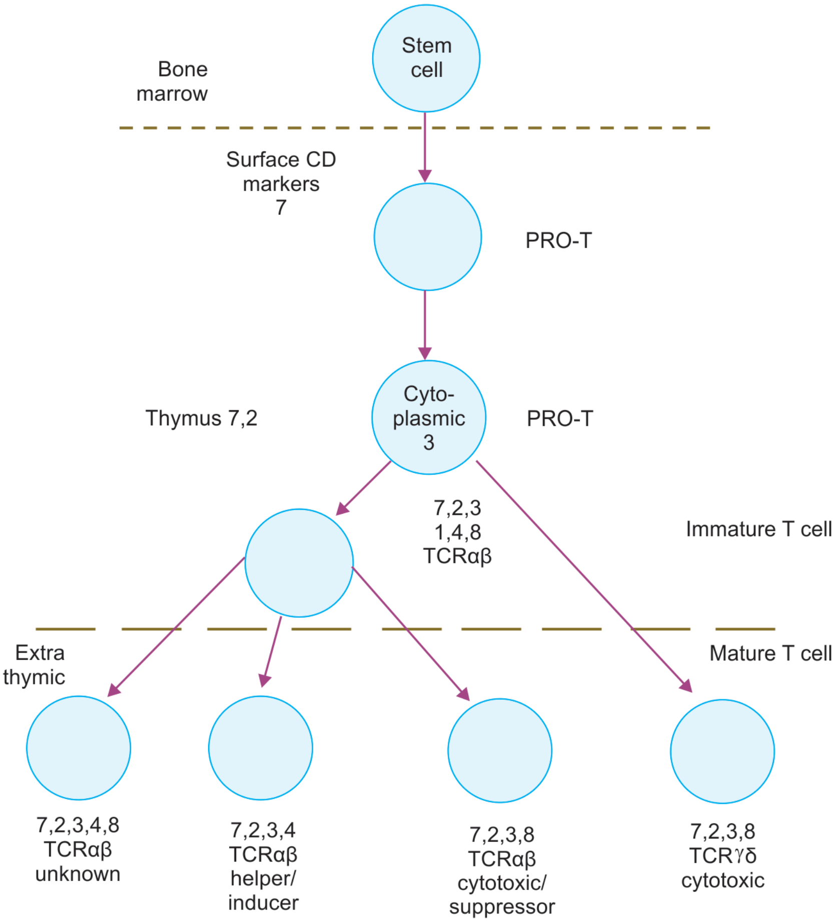

Bone marrow
Stem cell
Surface CD markers 7
PRO-T
Thymus 7,2
Cyto-plasmic 3
PRO-T
7,2,3
1,4,8
TCRαβ
Immature T cell
Extra thymic
Mature T cell
7,2,3,4,8
TCRαβ
unknown
7,2,3,4
TCRαβ
helper/
inducer
7,2,3,8
TCRαβ
cytotoxic/
suppressor
7,2,3,8
TCRγδ
cytotoxic

**Fig. 8.7:** T cell maturation

A unique subset of T cells have been found out, which shares the functional properties and the receptors of NK cells, developed in thymus and express a rearranged TCR with extremely limited repertoire. This is known as NKT cell. Unlike conventional T cells, NKT cells respond to lipids, glycolipids or hydrophilic peptides, presented by specialized non-classical MHC I molecule, CD1d and secrete large amount of cytokines specially IL-4.

A subpopulation of large granular lymphocytes (LGLs) possesses surface receptors (CD16 Fc receptor) for the Fc part of Ig molecule. They are capable of killing target cells sensitized with IgG antibodies. They are known as ADCC cells, which were formerly called killer cells ('K' cells). NK by contrast, occurs independently without antibodies.

When NK cells are stimulated by IL-2 produced by T helper cells, their cytotoxic effect is enhanced. The LAK cells are used clinically to treat tumor (renal cells carcinoma). Activation by IL-2 induces NK cells to express NKp44 in activated NK cells, activated

**Table 8.3** Distinguishing features of T cells, B cells and macrophages

| Property | T cells | B cells | Macrophages |
|---|---|---|---|
| CD3 receptor | + | – | – |
| Surface Igs | – | – | + |
| Receptor for Fc piece of Igs | – | + | (+/–) |
| Erythrocytes-antibody-complement (EAC) rosette (C3 receptor, CR2, Epstein-Barr virus receptor) | – | + | – |
| Sheep red blood cells rosette (CD2; measles receptor) | + | – | – |
| Thymus-specific antigen | + | – | – |
| Microvilli on surface | – | + | – |
| Blast formation with | | | |
| a. Anti-CD3 | + | – | – |
| b. Anti-Ig | – | + | – |
| c. Phytohemagglutinin | + | + | – |
| d. Concanavalin | + | – | – |
| e. Endotoxin | – | + | – |
| Phagocytic action | – | – | + |
| Adherence to glass surface | – | – | + |

NK cells produce cytokines such as interferon-gamma (IFN-$\gamma$), TNF-$\alpha$, granulocyte-macrophage colony-stimulating factor (GM-CSF) and CSF-1.

## Phagocytic Cells

Phagocytosis, phylogenetically, is the oldest defense mechanism in animals. In protozoa, both the functions such as nutrition and defense are performed by phagocytic cells. As an evolutionary process, phagocytes lost its trophic functions with the development of digestive system. In higher organisms the phagocytic cells remove effete and foreign particle. The phagocytic cells of the body may be as follows:

1. Macrophages.
2. Neutrophils.
3. Eosinophils.
4. Dendritic cells.

### *Macrophages*

The macrophages in the blood are known as monocytes or blood macrophages. The monocytes leave the circulation and reach various tissues and become transformed, to macrophages with morphological and functional features characteristic of the tissue, such as Kupffer cells in liver, alveolar macrophages in the lungs, Langerhans' cells in skin, osteoclasts in bone, etc. These macrophages proliferate and survive for months.

*Functions:* Macrophages perform following functions:

*Phagocytic response:* The primary function of the macrophages is phagocytosis. They are attracted to the area of inflammation and tissue damage by chemotaxis. Foreign substances, which are sensitized by antibody (opsonin) are phagocytosed more readily. The phagocytosed particles are taken inside the vacuole (phagosome), the membrane of which fuses with the lysosome called phagolysosome. Lysosomal enzymes digest the particle, the remnant being extruded from the cells. While, phagocytosis is an effective defense against most of the organisms, bacteria such as typhoid bacilli, brucellae and tubercle bacilli resist digestion and multiply inside the cells and are transported in them to other locations. Many stimuli can increase the functional activities of the macrophage. The important stimuli are as follows:

1. Direct contact with microorganism or their inner products such as endotoxin.
2. Protein components of complement or blood coagulation systems.
3. Interferon-γ produced by the adjacent T lymphocytes. Activated macrophages are metabolically active, which engulf the particles more readily than the ordinary macrophages. Like neutrophils, macrophages also recognize the target particles directly by their surface properties. However the cells also express receptors for complement components, Igs (opsonins). The coating of opsonin is important for phagocytosis.

*Antigen processing:* Antigen is processed in the macrophage and the processed antigen is presented along with major histocompatibility complex (MHC) molecule for attraction of T lymphocytes with complementary receptors on their surface.

*Cytokine production:* A number of biologically active substances such as hydrolytic enzymes, binding proteins (fibronectin, transferrin), TNF (cachectin), CSF, IL-1 are secreted by macrophages. IL-1 acts as endogenous pyrogen and also induces synthesis of IL-2 by activated T cells.

### *Neutrophils*

Neutrophils are actively phagocytic and form the important cell type in acute inflammation. The phagocytic property of neutrophil is non-specific; hence they are mostly the cells of the innate immunity except their augmentation by opsonin.

### *Eosinophils*

Eosinophils are found in large number, in allergic inflammation, parasitic infections and around antigen-antibody complex. Human eosinophils are slightly larger than neutrophils, being 12 to 17 µm in diameter. They con-tain fewer specific granules. Their major contents of the granules include eosinophilic peroxidase and other enzymes that can generate toxic metabolites and a cytotoxic lipophosphatase called Charcot-Leyden crystal protein and other basic proteins. One of them is major basic protein (MBP), which is toxic and cytolytic to larger parasites such as *Trichinella spiralis, Schistosomes, Fasciola* and filarial worms. They also can phagocytose many types of parasites, *in vitro* including bacteria, fungi, *Mycoplasma* and antigen-antibody complex. The eosinophils attach themselves tightly to the antibody coated or complement coated particles and discharge their granules contents on to its surface by extracellular degranulation.

*Basophils:* They are found in blood and tissues (mast cells). Their cytoplasm possesses large number of granules, which contain heparin, histamine, serotonin and other hydrolytic enzymes. Degranulation leads to anaphylaxis and atopic allergy.

### *Dendritic Cells*

Dendritic cells are the class of APCs that initiate most immune responses. They are found in nearly all tissues including surface epithelia and T cell zone of lymphoid tissue and have a spidery cytoplasmic projections called dendrites. The dendritic cells are smaller than the macrophage. They are not phagocytic and have no complement receptors, but they do possess class II antigen.

These cells are often seen after antigenic stimulation, being surrounded by lymphocytes and are involved in the immune response.

*Langerhans' cell:* They are a subpopulation of antigen-presenting dendritic cells present in the epidermis. They constitute about 5 percent of all epidermal cells. They are believed to process and present antigens that reach the dermis.

## MAJOR HISTOCOMPATIBILITY COMPLEX

The MHC was first detected, as the genetic locus encoding the glycoprotein molecules (transplantation antigens) responsible for the rapid rejection of the tissue graft. Subsequently, it was also known that it is a part of the genome that codes for molecules that are important in immune recognition.

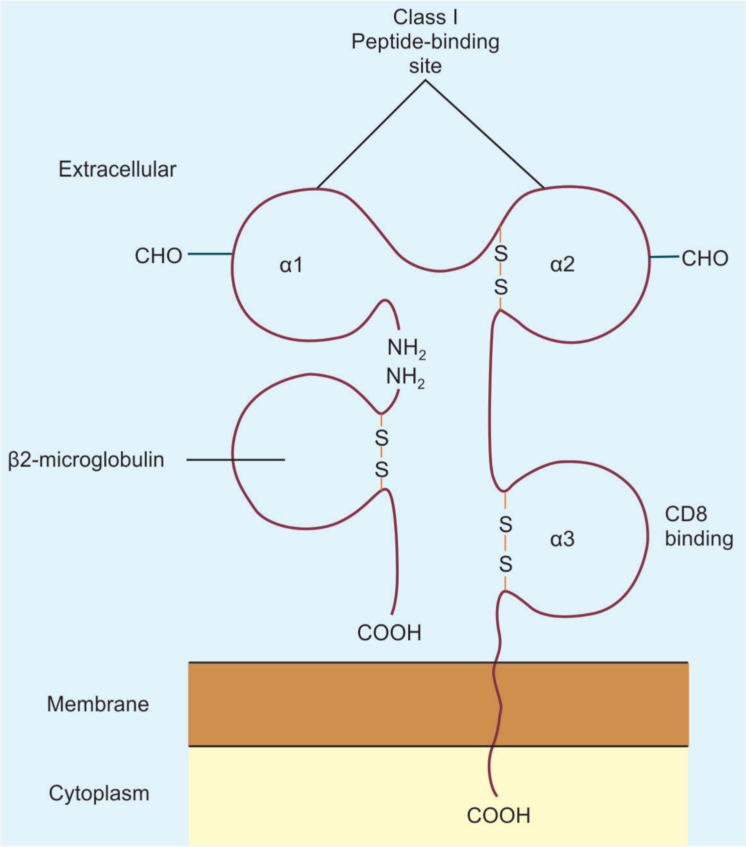

Class I
Peptide-binding
site
Extracellular
CHO
α1
S
S
α2
CHO
NH$_2$
NH$_2$
β2-microglobulin
S
S
S
S
α3
CD8
binding
COOH
Membrane
Cytoplasm
COOH

**Fig. 8.8:** Schematic representation of MHC class I: The class I molecule consists of a MW 43 kD polymorphic transmembrane polypeptide (chain) non-covalently associated with a MW 11 kD non-polymorphic polypeptide β2-microglobulin that is not anchored in the α-chain are designated α1, α2 and α3. The binding site for antigenic peptides is formed by the cleft between the α1 and α2 domains; CD8 contacts a portion of the α3 domain.

The MHC of mouse and human being, both have been studied most. The gene complex contains a large number of individual genes that can be grouped into three classes, on the basis of the structures and functions of their products. The products of the genes are referred to as MHC molecules or antigens. The MHC of human is known as human leukocyte antigen (HLA) and in mice it is referred to as histocompatibility-2 (H-2) (Fig. 8.8).

## Gene Organization

Genes that code for HLA antigens are found in the short arm of chromosome 6. They contain enough deoxyribonucleic acid (DNA) for over 200 genes. *MHC* genes are contained within regions known as A, B, C and D.

### *Class I Molecule*

A single gene that codes for the larger chain of the class I molecule is present in the A, B and C regions, while the lighter chain

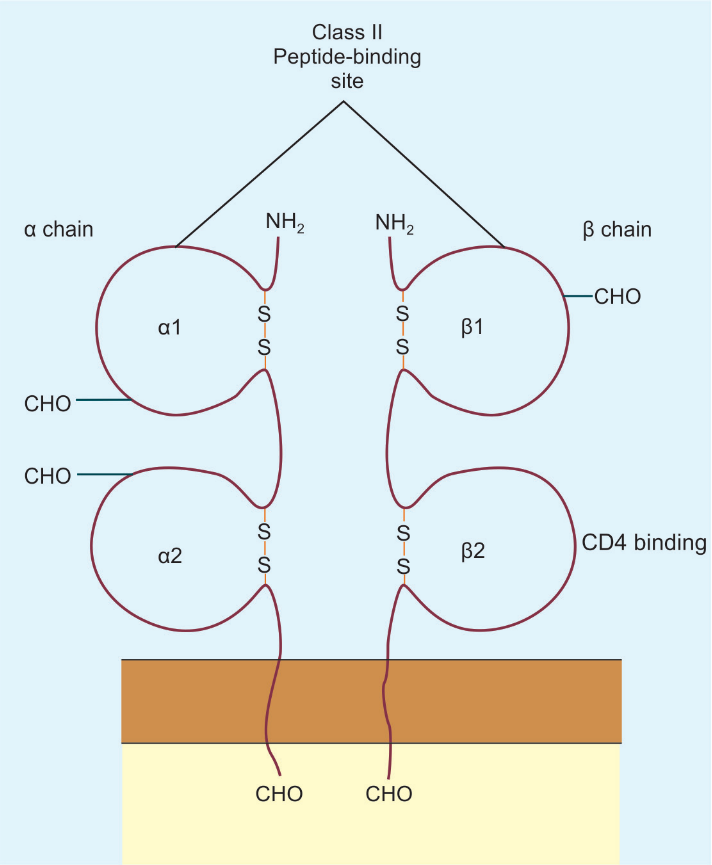


**Fig. 8.9:** Schematic representations of MHC class II: The class II molecule consists of a MW 34 kD α chain non-covalently associated with a MW 29 kD β chain, both of which are polymorphic. Antigen peptides bind a cleft formed by the α1 and β2 domains; CD4 contacts sequences in the β2 domains. Locations shown for the peptide and CD4 binding sites are approximations only.

β2-microglobulin is coded for elsewhere in the genome. MHC class I molecule consists of a heavy peptide chain of 43 kD non-covalently linked to a smaller 11 kD peptide called β2-microglobulin. The part of the heavy chain is organized into three globular domains (α1, α2 and α3), which protrude from the cell surface. The hydrophobic protein anchors the molecule on the cell membrane and a short hydrophilic sequence carries the COOH protein into the cytoplasm. Class I molecules are virtually present in all cells except the villous trophoblasts (Fig. 8.8).

### *Class II Molecule*

The MHC class II molecules are also transmembrane glycoproteins consisting of α and β peptide chains of molecular weight 34 kD and 29 kD respectively. Both chains are folded to give two domains (α1, α2 and β1, β2) (Fig. 8.9). The class II molecules are coded for within the D region. There are three class II molecules such as DP, DQ and DR (Table 8.4). The class II molecules are particularly associated with B cells and macrophages, but can be induced on capillary endothelial cells by γ-interferon.

### *Class III Molecule*

Class III genes are grouped together in a region between D and B. These genes code for number of complement components, TNF, heat shock protein (HSP), etc.

Human leukocyte antigen loci are multiallelic that is the gene occupying the locus can be any one of several alternative forms (alleles). As each allele determines a distinct product (antigen) HLA system is very pleomorphic. For example, at least 24 distinct alleles have been identified in HLA locus ‘A’ and 50 at B. The immune response (Ir) genes,

**Table 8.4** Simplified organization of the major histocompatibility complex (MHC) in the mouse and human

| Feature | Comparison | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Mouse H-2 complex** | | | | | | | | |
| Complex | H-2 | | | | | | | |
| MHC class | I | II | | III | | I | | |
| Region | k | IA | IE | S | | D | | |
| Gene products | H-2K | IA<br>αβ | IE<br>αβ | C proteins | TNF-α<br>TNF-β | H-2D | H-2L | |
| **Human MHC** | | | | | | | | |
| Complex | HLA | | | | | | | |
| MHC class | II | | | III | | I | | |
| Region | DP | DQ | DR | C4, C2, BF | | B | C | A |
| Gene products | DP<br>αβ | DQ<br>αβ | DR<br>αβ | C proteins | TNF-α<br>TNF-β | HLA-B | HLA-C | HLA-A |

The MHC is referred to as the H-2 complex in mice and as the HLA complex in humans. In both species, the MHC is organized into a number of regions encoding class I, class II and class III gene products. The class I and class II gene products shown in this figure are considered to be the classical MHC molecules. The class III gene products include complement (C′) proteins and the tumor necrosis factors (TNF-α and TNF-β).

which control immunological response are believed to be situated in HLA class II region, probably associated with direct repeat (DR) locus. HLA-DR antigens are known to generate strongest rejection reactions.

The MHC also contains many other genes that have no role in immunology, such as those encoding heat shock protein 2 or the steroidogenic enzyme 21-β hydroxylase. The functional significance of the latter's association, if any, is unknown.

## STUDY QUESTIONS

### Essay Questions

1. How do lymphoid stem cells become B cells and T cells?
2. How can the lymphocytes be distinguished?
3. Discuss the immune system and their function.

### Short Notes

1. T lymphocytes.
2. B lymphocytes.
3. NK cells.
4. Interferon.
5. Histocompatibility antigen.
6. Major histocompatibility complex.

## SUGGESTED READING

1. Black JG. Microbiology principles and applications, 3rd edition; 1996.
2. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology, New York: Wiley; 1997.
3. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
4. Greenwood D, Slack R. Medical microbiology, 15th edition; 1997.
5. Jawetz. Textbook of microbiology, 25th edition; 2010.
6. Male David, Brostoff Jonathan, Roth David B. Immunology, 7th edition; 2006.
7. Stites. Basic and clinical immunology, 8th edition; 2007.
8. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
9. Tortora, Funke, Case. Microbiology an introduction, 6th edition; 1998.

# 9

# Immune Response

The protective reactions underlying acquired immunity are called immune responses. The acquired immune system differs considerably from innate systems in its interaction with the pathogens. Innate immunity mainly recognizes carbohydrates, lipids and N-formylated peptides that are foreign, but acquired immune responses most commonly, directed against proteins, a class of molecules found both in pathogens and hosts. The acquired immune system discriminates between self and non-self, so that it normally coexists peacefully with the proteins and other organic materials that make-up the host, but responds vigorously against foreign organisms.

Every immune response is a complex and intricately regulated sequence of events involving several cell types. The immune response starts, when antigen enters the body and encounters a specialized class of cells called antigen-presenting cells (APC).

## ANTIGEN PROCESSING AND PRESENTATION

Immune responses to proteinaceous antigens, begin when they are captured by the APCs—(macrophage, dendritic cells, B cells) processed and presented with major histocompatibility complex (MHC) molecule. The reason is that T cells only recognize immunogens that are bound to MHC. There are two different classes of MHC proteins, which are recognized by two subsets of T lymphocytes. Class I MHC proteins, are expressed in all the cells of the body and are recognized by cluster of differentiation (CD8) T cells of which most are cytotoxic. Therefore, any cell can present antigen with MHC I protein and may be the target of cytotoxic action of CD8 cells. On the other hand, MHC II proteins are present in macrophages and few other cells, which along with the processed antigen invite CD4 (helper) subset of T cells. Since, helper cells activation is essential for most of the immune responses, MHC II bearing APCs play a vital role in controlling immune responses. These APCs include dendritic cells, macrophages and B cells.

### Exogenous Antigen

Exogenous antigen (bacteria, virus, etc.) enters the cell by endocytosis or phagocytosis (Fig. 9.1). APC (macrophages, dendritic cells, B cells) degrade ingested exogenous antigens into smaller peptides (10–25 amino acids) within the endocytic processing pathway. The class II MHC molecules are synthesized in the rough endoplasmic reticulum. The peptides produced by degradation of antigen bind to the cleft within the class II MHC molecule. The MHC molecules bearing the peptide are then exported to the cell surface. Since, expression of class II MHC molecules is limited to APCs, presentation of exogenous peptide-class II MHC complexes is limited to these cells. T cells displaying CD4, recognize antigen combined with class II MHC molecules and therefore, they are said to be class II MHC restricted (Fig. 9.1A).

## Endogenous Antigen

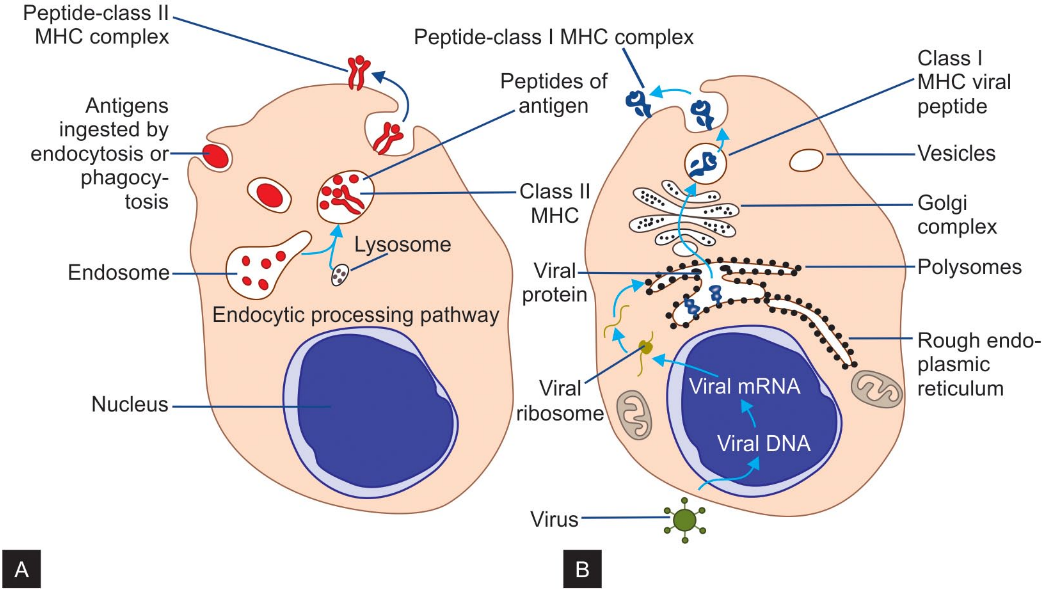
Endogenous antigen is produced within the host cell itself. Two common examples are:

1. Viral protein synthesized in virus infected host cells.
2. Unique protein synthesis of cancerous cells.

Endogenous antigens are thought to be degraded into peptide fragments (9–12 amino acids) that bind to class I MHC molecule within the endoplasmic reticulum. The peptide-MHC I complexes are then transported to the cell surface. Since, all nucleated cells of the body express class I molecules, all cells producing endogenous antigen use this route of processing the antigen. T cells displaying CD8 receptor [cytotoxic T cells ($T_c$)] recognize the peptide-MHC I complex and are said to be class I MHC restricted. These $T_c$ attack and kill cells, displaying the antigen-MHC I complexes for which their receptors are specific (Fig. 9.1B).

The binding groove of the class I molecule is more constrained, to accommodate only few shorter peptides than the class II, which can accommodate longer peptides.

A detail understanding about the antigen processing has clarified our doubts, as to why


Peptide-class II MHC complex
Antigens ingested by endocytosis or phagocytosis
Endosome
Lysosome
Endocytic processing pathway
Nucleus
Peptides of antigen
Class II MHC
A
Peptide-class I MHC complex
Class I MHC viral peptide
Vesicles
Golgi complex
Polysomes
Viral protein
Rough endoplasmic reticulum
Viral ribosome
Viral mRNA
Viral DNA
Virus
B

**Figs 9.1A and B:** Processing and presentation of exogenous and endogenous antigens. **A.** Exogenous antigen is ingested by endocytosis or phagocytosis and then enters the endocytic processing pathway. Here, within an acidic environment, the antigen is degraded into small peptides, which are presented with class II MHC molecules on the membrane of the antigen-presenting cell; **B.** Endogenous antigen, which is produced within the cytoplasm into peptides, which move into the endoplasmic reticulum, where they bind to class I MHC molecules. The peptide-class I MHC complexes then move through the Golgi complex to the cell surface.

carbohydrate antigens fail to attract T cells in contrast to their protein counter parts. Possibly, it is because that the carbohydrate antigens do not fit well into the groove of MHC, where as the processed protein antigens are arranged in a linear fashion to fit exactly, into the groove of MHC.

There are certain antigens, which are designated as superantigens, which bind the MHC molecule outside the peptide binding cleft (Fig. 9.2), causing activation of a good number of T cells (10% of all T cells), releasing large amount of cytokines including interleukin-1 (IL-1) and tumor necrosis factor (TNF). Excessive production of cytokines from high percentage of pool of T lymphocytes explain to a large extent, the pathogenesis of the disease caused by the organisms with superantigens (staphylococcal enterotoxin, toxic shock syndrome toxin and group 'A' streptococcal pyrogenic toxin).

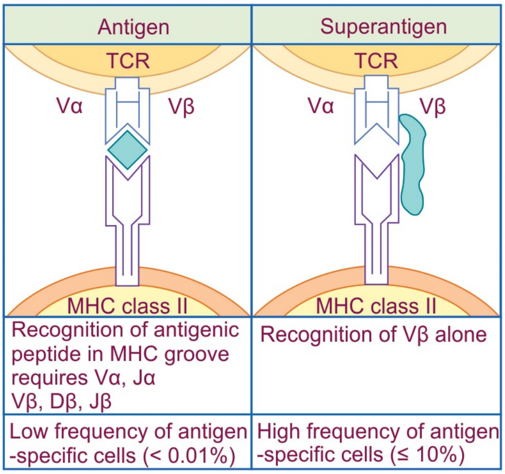

Antigen
Superantigen
TCR
TCR
Vα
Vβ
Vα
Vβ
MHC class II
MHC class II
Recognition of antigenic peptide in MHC groove requires Vα, Jα Vβ, Dβ, Jβ
Recognition of Vβ alone
Low frequency of antigen -specific cells (< 0.01%)
High frequency of antigen -specific cells (≤ 10%)

**Fig. 9.2:** Antigenic peptides must normally be processed in order to trigger the TCR. However, superantigens such as staphylococcal enterotoxins are not processed, but bind directly to MHC class II and Vβ. Each superantigen activates a distinct set of Vβ expressing T cells. This depends on which Vβ gene segment the T cell is using to encode its receptor.

## ACTIVATION OF HELPER T LYMPHOCYTES

Activation of helper T lymphocytes is of utmost importance in the initiation and ongoing of immune response. Its activation leads to activation of two other lymphoid effector cell types. They are $T_c$ cells and antibody-secreting plasma cell. T helper ($T_h$) cell activation is initiated by interaction of T-cell receptor (TCR)-CD3 complex with a processed antigenic peptide bound to a class II MHC molecule on the surface of APC. This interaction and resulting activation signal also involve various accessory membrane molecules on the $T_h$ cell [leukocyte function antigen, (LFA-1), CD2] and the APC [intercelluar adhesion molecule (ICAM-1), LFA-3]. LFA-1 of $T_h$ cell interacts with ICAM-1 of APC. Simultaneously, CD2 of $T_h$ cell reacts with LFA-3 of APC (Fig. 9.3). Interaction of $T_h$ cells with antigen, initiates a cascade of biochemical events that induces the resting $T_h$ cell, to enter the cell cycle, proliferating and differentiating into memory cell or effector cell. The changes that occur are the result of signal transduction pathways that are activated by the encounter between TCR and MHC-peptide complex.

In addition to the interaction of antigenic peptide with TCR-CD3 complex (signal 1), costimulatory signals are required for full T cells activation. A subsequent antigen-non-specific costimulatory signal (signal 2), is provided by the interaction of CD28 on the T cell with the B-7 family on the APC (Refer Fig. 9.9). The presence or absence of costimulatory signal (signal 2) determines, whether activation results in clonal expansion or clonal anergy.

Both the signals induce the ($T_h$ cells) to produce IL-2, which have got actions on the cells, which have produced autocrine effects and action on the cells and in the vicinity, paracrine effect. Due to autocrine effect,

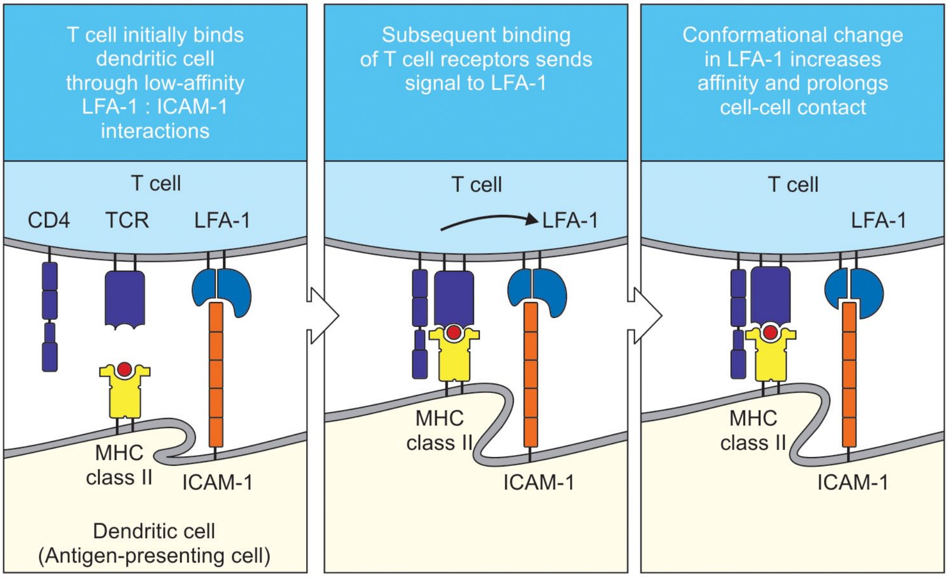

T cell initially binds dendritic cell through low-affinity LFA-1 : ICAM-1 interactions
Subsequent binding of T cell receptors sends signal to LFA-1
Conformational change in LFA-1 increases affinity and prolongs cell-cell contact
T cell
CD4
TCR
LFA-1
MHC class II
ICAM-1
Dendritic cell (Antigen-presenting cell)
T cell
LFA-1
MHC class II
ICAM-1
T cell
LFA-1
MHC class II
ICAM-1

**Fig. 9.3:** Activation of naive T cells on encounter with antigen (LFA, leukocyte function antigen; ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; TCR, T cell receptor).

there is appearance of IL-2 receptors on the $T_h$ cells. The presence of IL-2 receptors is essential for proliferation. IL-2 secreted by $T_h$ cells also has paracrine effect. This is important for activating $T_c$ cells. In addition to IL-2, activated $T_h$ cells secrete other cytokines that promote the growth, differentiation and proliferation of B cells, macrophages and other cell types (Fig. 9.4).

## Activation of Cytotoxic T Cells

Most of the $T_c$ cells express CD8 molecule rather than CD4 on their surface. The $T_c$ cell, recognize the antigenic peptide (virus infected cell) in association with MHC I molecule. The peptide-MHC complex forms the first signal for the activation of $T_c$ cells. The first signal induces high affinity IL-2 receptors on the T cell. The second signal is offered by IL-2 secreted by nearby activated $T_h$ cell. APC-CD4 T cell interaction, increases CD80/86 expressions by APC. Interaction of APC CD80/86 with CD28 on CD8 T cells promotes CD8 T cells differentiation. CD8 T cells differentiate into cytolytic effector cells called cytotoxic T lymphocytes. On receiving both the signals, activated $T_c$ cells acquire cytotoxic property, killing the target cells. Destruction occurs by release of two types of cytolytic granules, perforin (a pore forming protein) and granzymes (serine proteases) into the target cell causing suicide by apoptosis. Apoptosis is a programmed cell death caused by intense activation of T and B cells (activation-induced cell death) (Fig. 9.5).

## Activation and Proliferation of B Cells

After formation on the bone marrow, B cells are exported to periphery for activation and proliferation following contact with antigen. Antigen-driven activation and clonal selection leads to conversion to plasma cells and memory B cells. Virgin B cells (not activated by antigen) have a short lifespan and die earlier.

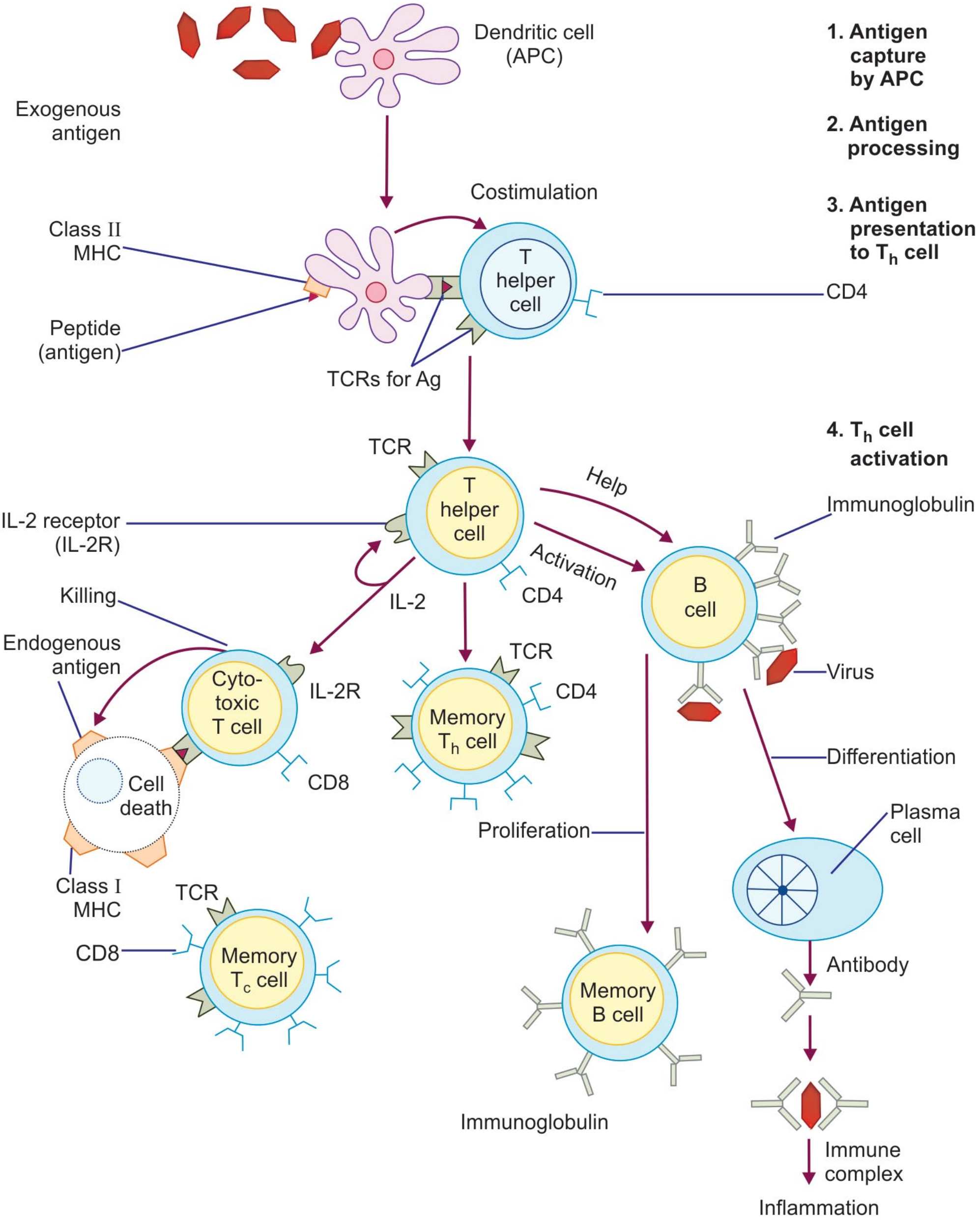

Dendritic cell
(APC)
1. Antigen capture by APC
2. Antigen processing
3. Antigen presentation to $T_h$ cell
4. $T_h$ cell activation
Exogenous antigen
Costimulation
Class II MHC
T helper cell
Peptide (antigen)
CD4
TCRs for Ag
TCR
T helper cell
Help
Immunoglobulin
IL-2 receptor (IL-2R)
Activation
B cell
IL-2
CD4
Killing
Endogenous antigen
Cyto-toxic T cell
IL-2R
TCR
CD4
Memory $T_h$ cell
Virus
Cell death
CD8
Differentiation
Proliferation
Plasma cell
Class I MHC
TCR
CD8
Memory $T_c$ cell
Memory B cell
Antibody
Immunoglobulin
Immune complex
Inflammation

Fig. 9.4: Sequence of events in a prototypical immune response (MHC, major histocompatibility complex; APC, antigen-presenting cell; TCR, T cell receptor).

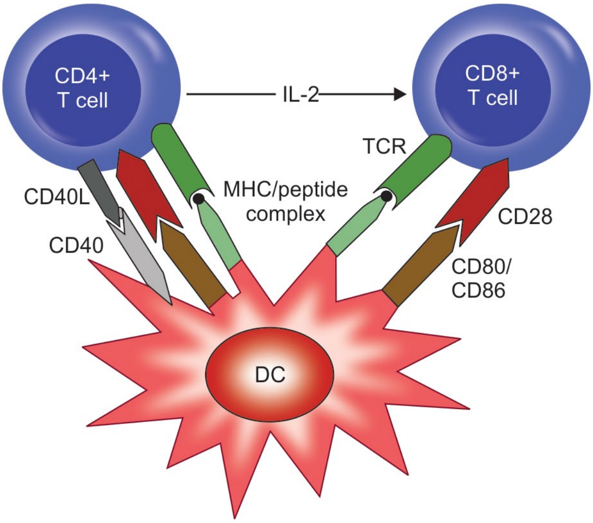


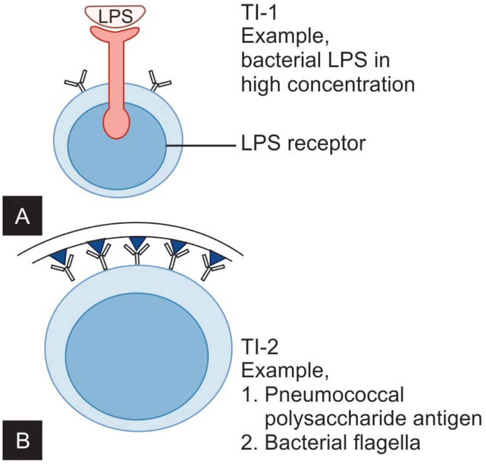
**Fig. 9.5:** T cell ($CD4^+$ and $CD8^+$) activation (TCR, T cell receptor; DC, dendrite cell).

The activation and proliferation of B cell depends upon the types of antigens, T-independent (TI-1 and TI-2) antigens or T-dependent antigens. These two types of antigens have different requirements for the response.

### T-independent Activation of B Cells

*T (independent-1) activation of B cells:* The name indicates that the antigens, under this group, do not need the help of T cells for activation of B cells. T-independent antigens are of two distinct groups (TI-1 and TI-2), based upon how they activate B cells. TI-1 antigens are polyclonal activators (mitogens) that bind to surface structure other than B cell receptors (BCRs). Therefore, they activate B cells regardless of their BCR epitope specificity. Some bacterial cell wall components including lipopolysaccharide (LPS) function as TI-1 antigens. In high concentration, TI-1 antigens will stimulate proliferation and antibody secretion by one-third of all B cells. The mechanism how TI-1 antigen activate B cell is not well understood, but they stimulate both mature and immature B cells (Fig. 9.6A).

*T (independent-2) activation of B cells:* TI-2 antigens specifically activate only mature cells. TI-2 antigens activate B cells by extensively cross-linking the membrane immunoglobulin (mIg) BCRs. TI-2 antigens are highly repetitive molecules, such as polymeric proteins (e.g. bacterial flagellin) or bacterial cell wall polysaccharides with repeating epitopes (Fig. 9.6B). Although, the B cell response to TI-2 antigen does not require direct involvement of T cells, the cytokines produced by $T_h$ cells are required for efficient B cell proliferation and for class switching to isotypes other than IgM. Following cross-linking of specific BCRs, the close proximity of Igα and Igβ cytoplasmic tail result in phosphor relation and initiation of signal transduction cascade. Sometimes B cell coreceptor complex, in conjunction with complements induces T-independent signal for B-cell activation (Fig. 9.7). C3d or C3b binds to an antigen (e.g. bacteria) that also bound to the BCR through epitope recognition. CD21 (CR2) binds to antigen-bound C3b. Cell membrane bound CD19 and CD81



**Figs 9.6A and B:** B cell activation by T-independent antigens (T1-Ags). **A.** TI-1 Ags activate B cells by signaling primarily through non-immunoglobulin receptors, although specific surface antibodies can enhance signaling by concentrating the antigen on the cell surface. A hypothetical receptor for lipopolysaccharide (LPS) is shown; **B.** TI-2 antigens are highly repetitive structures and therefore, can activate B cells by specifically binding and cross-linking numerous surface immunoglobulins.

or target of an antiproliferative antibody (TAPA1) rapidly associate to form B-cell coreceptor complex (CD21:CD19:CD81). Once B-cell coreceptor complex is formed several tyrosine kinases (lyn or fyn and vav or PI-3k) phosphorylase the cytoplasmic tail of CD19. At the same time, fyn, lyn and/or blk phosphorylate, immunoreceptor tyrosine-based activation motifs (ITAM) on Igα and Igβ to allow the ducking of syk tyrosine kinase and the initiation of signal transduction cascade (Fig. 9.8).

### *T-dependent Activation of B Cells*

Activation of B cells by soluble protein antigens requires the help of T cells. Simply binding of the antigen to the B cells mIg does not induce an effective signal unless additional interactions with $T_h$ cell takes place. In addition, cytokines are also required for B-cell proliferation.

### Stages of Activation and Proliferation of B Cells

1. Signal 1 is generated by antigen cross-linking to mIg on BCR, increasing expression of class II MHC and costimulatory B7 molecules. Antigen-antibody are internalized and degraded to peptides. Some of the peptides are bound by MHC II and/or presented as peptide-MHC II (p-MHC II).
2. Once the $T_h$ cell recognizes p-MHC II on membrane of a B cell, the two cells interact to form T-B conjugate. T-B conjugate along with costimulatory molecules signal activates $T_h$ cells.
3. $T_h$ cell starts expressing CD154 [CD40 ligand (CD 40L)], which interacts with CD40 of B cell. This provides the second signal 2. This CD40-CD40L interaction is known as mediator of contact dependent help. The B7-CD28 interactions provide costimulation to $T_h$ cells.
4. Once activated, the B cells begin to express membrane receptors for various cytokines, such as IL-2, IL-4, IL-5 and others. These receptors then bind the cytokines produced by the interacting $T_h$ cell. The signals produced by these cytokine receptor interaction support B-cell proliferation and differentiation into plasma cell, memory B cell and class switching (Figs 9.9 and 9.10).

## HUMORAL IMMUNE RESPONSE (HUMORAL IMMUNITY)

Humoral immune response (Fig. 9.11) involves the production of large quantity of antibodies following B-cell activation, on antigenic challenge. The activation of B cells need the help of T cells in case of T-dependent antigens and may not require the help of T cells in case of TI antigens. The TI

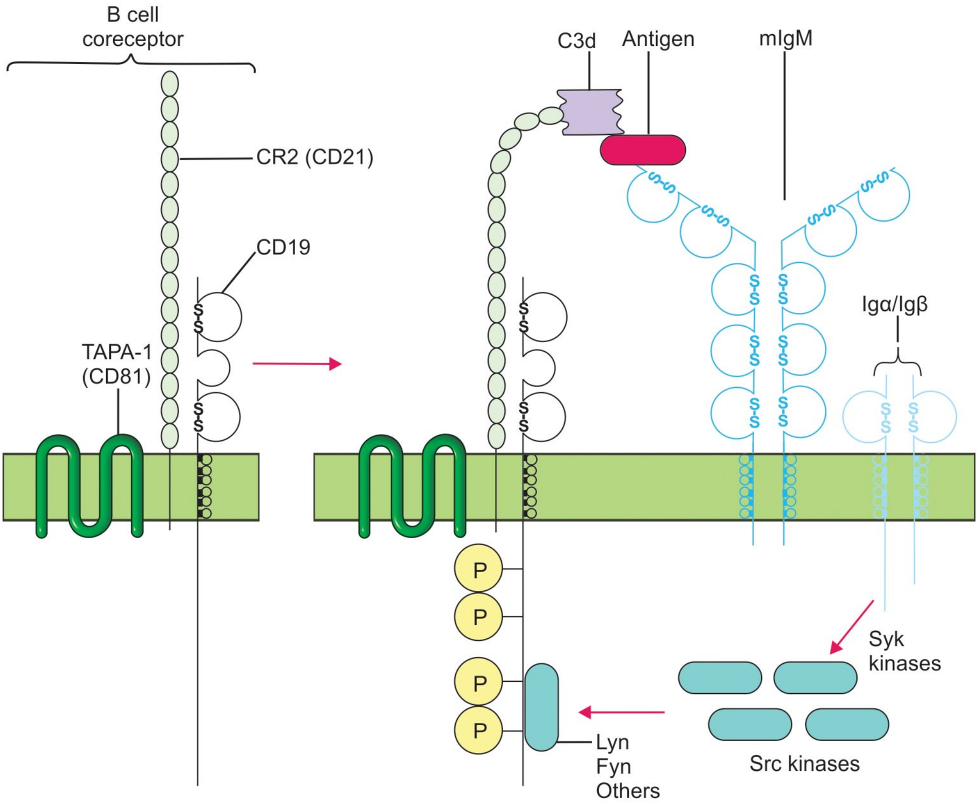


**Fig. 9.7:** The B cell coreceptor is a complex of three cell membrane molecules: TAPA-1 (CD81), CR2 (CD21) and CD19. Binding of the CR2 component to complement-derived C3d that has coated antigen captured by mIgM results in the phosphorylation of CD19. The Src family tyrosine kinase Lyn binds to phosphorylated CD19. The resulting activated Lyn and Fyn can trigger the signal transduction pathways.

antigens fall into two groups such as TI-1 antigens and TI-2 antigens. TI-1 antigens, in high concentration, induce activation of many B cells, both specific and non-specific. Because they activate many B cells, they are known as polyclonal B-cell activators. Many polyclonal activators (LPS) also activate macrophage to produce IL-1 and TNF-α, which augment immune responses. In contrast, TI-2 antigens are not polyclonal activators nor do they activate macrophage. Usually, these are highly repetitive polymeric antigens, such as polysaccharide from bacterial cell walls or polymeric protein, such as bacterial flagella. B-cell activating properties in TI-2 antigen may be due to cross-linking of numerous BCR molecules inducing intracellular signaling reactions.

## Scope

Humoral immune response plays important role in the primary defense against extracellular bacterial pathogens. Antibodies that

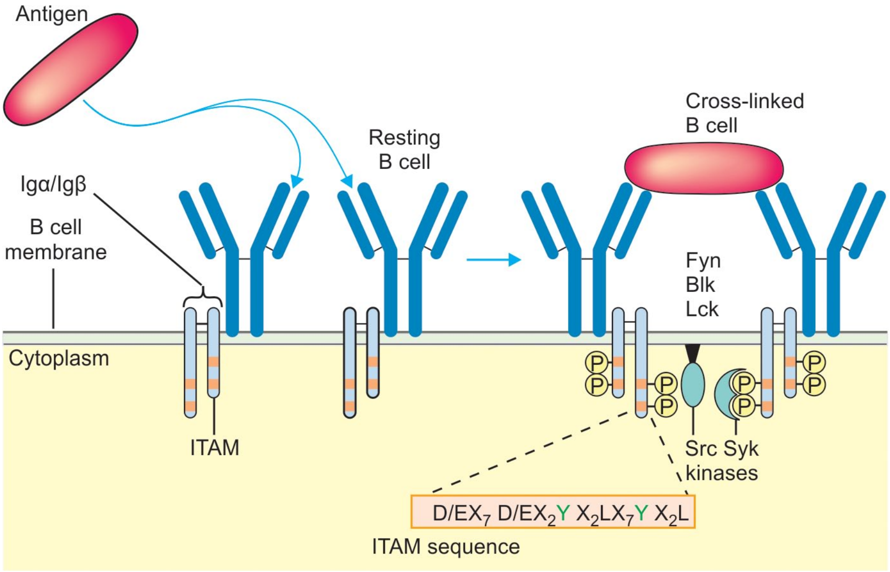
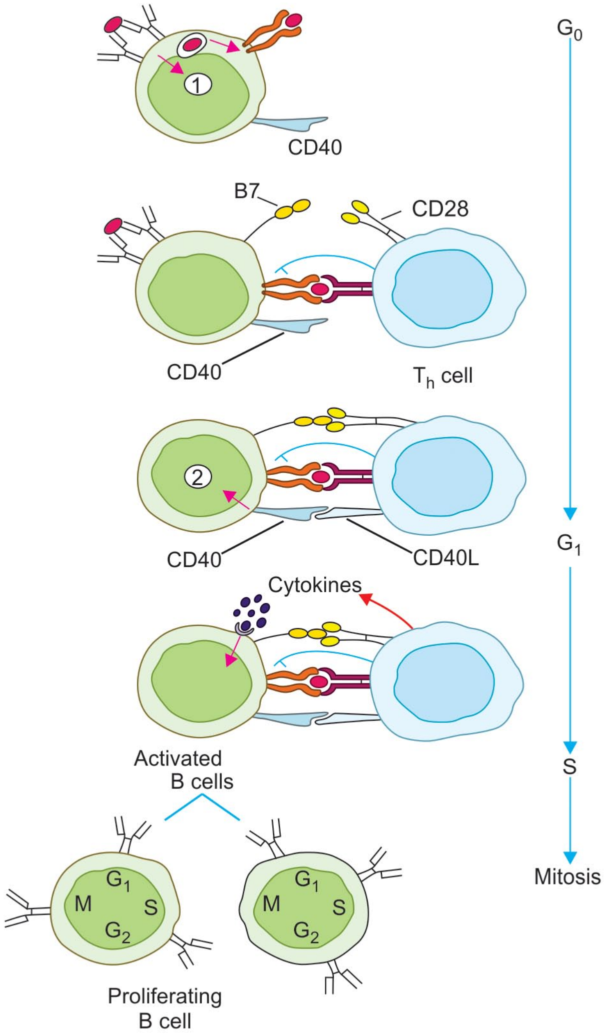


**Fig. 9.8:** The initial stages of signal transduction by an activated B cell receptor (BCR). The BCR comprises an antigen-binding mIg and one signal transducing Igα/Igβ heterodimer. Following antigen cross-linkage of the BCR, the immunoreceptor tyrosine-based activation motifs (ITAMs) interact with several members of the Src family of tyrosine kinases (Fyn, Blk and Lck), activating the kinases. The activated enzymes phosphorylated tyrosine residues on the cytoplasmic tails of the Igα/Igβ heterodimer, creating docking sites for Syk kinase, which is then also activated. The highly conserved sequence motif of ITAMs is shown with the tyrosines (Y) in green. D/E indicates that an aspartate or a glutamate can appear at the indicated position and X indicates that the position can be occupied by any amino acid.

coat bacteria or other particulate antigens can function as opsonin to promote phagocytosis. Antibodies specific for bacterial toxins or for the venom of the insects or snakes, bind these antigenic proteins and in many cases directly inactivate them by steric effects. In addition, the toxin-antitoxin complex may ultimately be phagocytosed by macrophages and other phagocytic cells. Certain types of antibodies, coated over the surface of the bacteria, can activate complement pathway leading to complement-mediated lysis. There is another mechanism, how antibodies play a role in causing cytolysis of bacteria and multicellular parasites, is by antibody-dependant cell-mediated cytotoxicity (ADCC). IgG binds Fc receptors on the surface of natural killer (NK) cells and certain other cell types and enables them to carry out a form of antigen-specific killing by cytotoxin.

Antibodies also bring about defense against viruses that infect through the respiratory tract and intestinal tract. Antibodies specific for protein on the surface of a virus may block the adsorption site on the target cell, thus preventing the entry of the organism.

Besides elimination of foreign substances (antigens), humoral immunity participates in the mechanism of pathogenesis of immediate hypersensitivity reaction and autoimmune diseases.



Antigen crosslinks mIg, generating signal ①, which leads to increased expression of class II MHC and costimulatory B7. Antigen-antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptices, some of which are bound by class II MHC and presented on the membrane as peptide-MHC complexes.

$T_h$ cell recognizes antigen-class II MHC on B-cell membrane. This plus costimulatory signal activates $T_h$ cell.

$T_h$ cell begins to express CD40L. Interaction of CD40 and CD40L provides signal②. B7-CD28 interactions provide costimulation to the $T_h$ cell.

B cell begins to express receptors for various cytokines. Binding of cytokines released from $T_h$ cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.

**Fig. 9.9:** Sequence of events in B cell activation by a thymus-dependent antigen. The cell cycle phase of the interacting B cell is indicated on the right (MHC, major histocompatibility complex; DNA, deoxyribonucleic acid).

## Sites for Induction

When antigen enters into the body, they are concentrated in various peripheral lymphoid tissues, which have been discussed in Chapter 8. Antigen from the tissue spaces, drained by the lymphatics and filtered by the lymph gland. Similarly, blood-borne antigen is filtered by the spleen. The following description, e.g. focuses on generation of immune response in lymph node.

Lymph node acts as an efficient filter and trap most of the antigens carried into it by the afferent lymphatics. Antigen or antigen-antibody complex come to lymph node alone or carried by transporting cells (e.g. Langerhans cell or dendritic cells) and macrophages. As

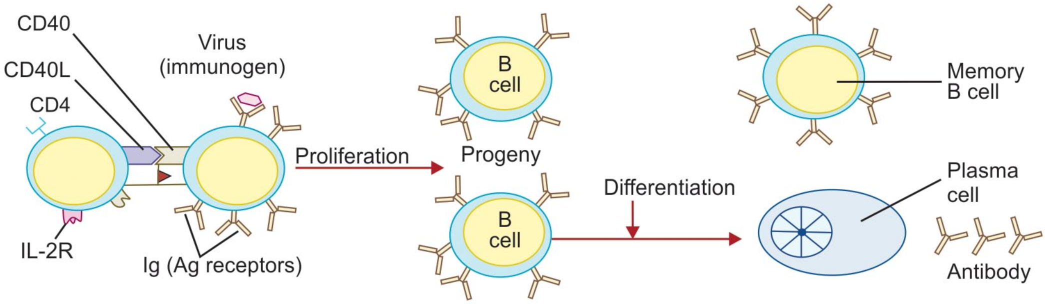

CD40
CD40L
CD4
Virus (immunogen)
IL-2R
Ig (Ag receptors)
Proliferation
B cell
Progeny
B cell
Differentiation
Memory B cell
Plasma cell
Antibody

**Fig. 9.10:** B cell activation. Antigen binding to the surface immunoglobulins, coupled with soluble or contact-mediated helper factors from an activated $T_h$ cell, lead to proliferation and differentiation, cytokines involved in $T_h$ cell help to include interleukin-2 (IL-2), IL-4 and IL-6. Contact-mediated help generally involves binding of CD40 on the B cell surface to CD40 ligand (CD40L) on the activated $T_h$ cell.

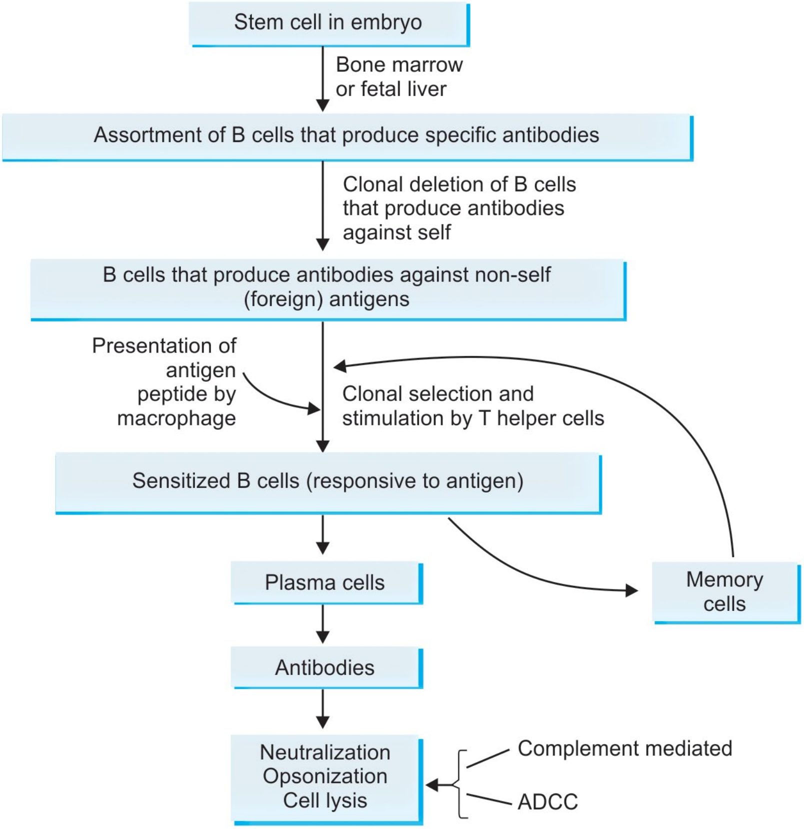

Stem cell in embryo
Bone marrow or fetal liver
Assortment of B cells that produce specific antibodies
Clonal deletion of B cells that produce antibodies against self
B cells that produce antibodies against non-self (foreign) antigens
Presentation of antigen peptide by macrophage
Clonal selection and stimulation by T helper cells
Sensitized B cells (responsive to antigen)
Plasma cells
Memory cells
Antibodies
Neutralization
Opsonization
Cell lysis
Complement mediated
ADCC

**Fig. 9.11:** Summary of humoral immunity (ADCC, antibody-dependent cell-mediated cytotoxicity).

the antigen passes through the cellular architecture of lymph node, it will encounter one of the three types of APCs interdigitating dendritic cells in paracortex, macrophages scattered throughout the follicular dendritic cells in the follicles and germinal centers. Antigenic challenge leading to humoral response involves complex series of events, which occur in the microenvironment of lymph node.

Slightly, different pathways may operate during primary and secondary response, because much of the tissue antigen is complexed with the circulating antibody in secondary response. In primary response, following antigen-mediated activation, small foci of B cells form at the edges of the T cell-rich zone. These B cells differentiate into plasma cells secreting IgM isotypes. A similar sequence of events take place in the spleen, where initial B cell activation takes place in T cell-rich periarterial lymphatic sheath (PALS).

It takes 7 to 10 days to develop germinal center, following initial exposure to T-dependent antigen. Following exposure, intense proliferation of B cells occur, which are known as centroblasts. These centroblasts appear as well-defined dark zone. The centroblasts are large sized cells with diffused chromatin with devoid or near absence of surface Ig. The centroblasts are converted to centrocytes, which are small and non-dividing B cells with surface Ig, which move from dark zone to a zone, which is known as lighter zone, which contains large number of follicular dendrite cells. In the lighter zone, the centrocytes make contact with antigen displayed as antigen-antibody complex on the surface of follicular dendritic cells. Three important B cell differentiations take place in the germinal center. They are affinity maturation, class switching from IgM to IgG and other isotypes and formation of plasma cells and memory cells.

### Kinetic and Quantitative Aspects

Antibody production follows a characteristic pattern after an initial antigenic challenge.

The pattern consists of:

1. A lag phase or latent phase, which lasts for 1 week in human beings during which no antibody is detected. During this period, activation of $T_h$ and B cells is taking place.
2. A log phase or exponential phase, where there is a steady rise of antibody titer. This phase results the increasing number of plasma cells.
3. A plateau or a steady state, where there is equilibrium between antibody synthesis and catabolism.
4. Phase of decline in which the antibody titer diminishes, which indicates that new plasma cells are no longer produced and the existing plasma cells are dying or ceasing antibody production.

## PRIMARY AND SECONDARY RESPONSE

The antibody response to an initial antigenic stimulus differs qualitatively and quantitatively from the response to the subsequent stimuli with the same antigen (Fig. 9.12).

An individual's first encounter with a particular immunogen leads to a relatively slow, sluggish short-lived response designated as primary response. There is a longer lag phase and the titer of antibody is low, which does not persist longer and the antibodies formed are predominantly IgM in nature.

In contrast, when the same individual encounters with the same immunogen subsequently, the response is prompt, powerful and prolonged, where there is no lag phase or shorter lag phase, antibody titer is high and persists for longer period and the predominant antibody formed is IgG. This is known as secondary or anamnestic

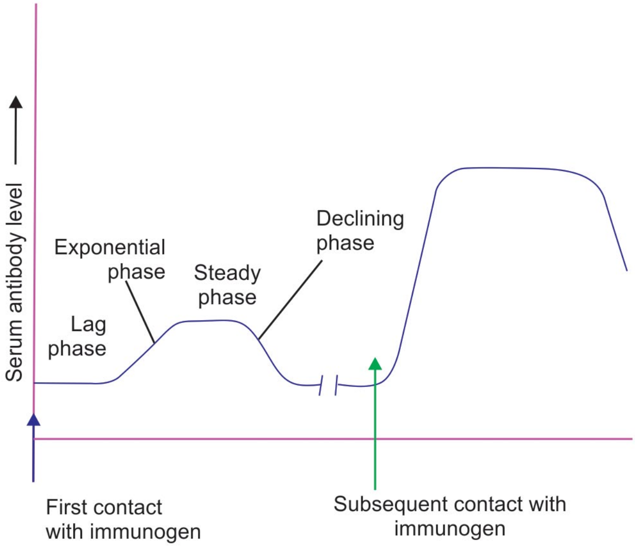


**Fig. 9.12:** Primary and secondary immune responses

reaction. The large numbers of antigen-specific memory T and B cells, generated during the primary response are responsible for the rapid kinetics and the greater intensity and duration of secondary responses.

## MONOCLONAL ANTIBODIES AND HYBRIDOMA TECHNIQUE

Antibodies have many applications in diagnosis therapy and research. Until, recently all the antibodies were produced by inoculating antigen into the animals and taking serum sometime later. One of the drawbacks of the conventional antisera is that, they are invariably a heterogeneous mixture of antibodies of different specificities, affinities and classes. A sought after antibody might be present at an inconveniently low concentration in serum. Further, because of its heterogeneity an antiserum might produce an unwelcome cross-reaction.

### Monoclonal Antibodies

Monoclonal antibodies are produced by a single clone of B cell, directed against a single antigenic determinant. The antibodies are monospecific, i.e. antibodies of one class with high affinity.

### Hybridoma Technique

In 1975, a method was devised by Kohler G and Milstein C in which monoclonal antibodies of any desire specificity could be manufactured in the laboratory. The technique is known as hybridoma technique. In recognition of this work, Nobel prize in medicine was awarded to them in 1984. In short, a B cell essentially be immortalized by fusion with a myeloma cell.

### Production

Monoclonal antibodies are useful for three reasons. They are uniform, can be highly specific and can be readily produced in large quantities for indefinite period (Fig. 9.13).

*Explanation for Figure 9.13*

1. A mouse is injected with a specific antigen that will produce antibodies against that antigen.

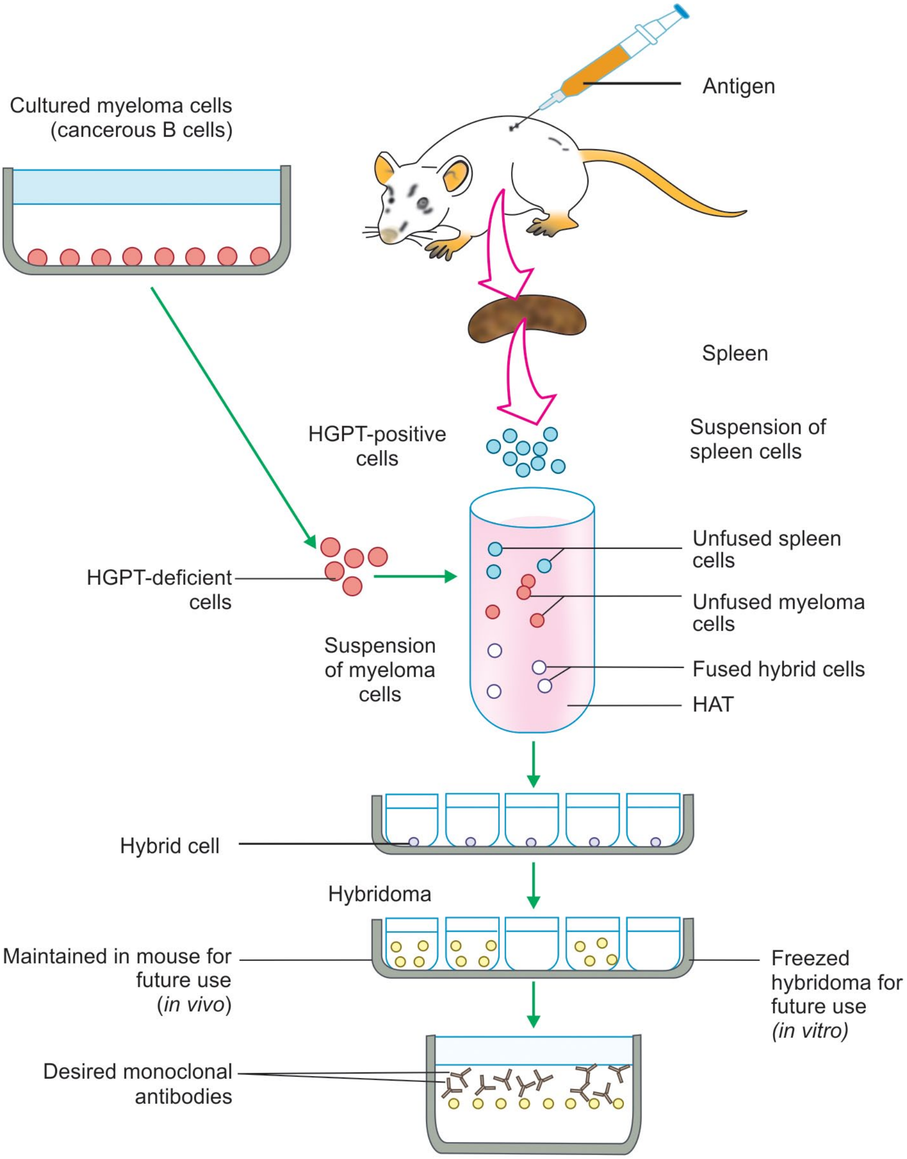

Antigen
Cultured myeloma cells
(cancerous B cells)
Spleen
HGPT-positive
cells
Suspension of
spleen cells
HGPT-deficient
cells
Unfused spleen
cells
Unfused myeloma
cells
Suspension
of myeloma
cells
Fused hybrid cells
HAT
Hybrid cell
Hybridoma
Maintained in mouse for
future use
(*in vivo*)
Freezed
hybridoma for
future use
(*in vitro*)
Desired monoclonal
antibodies

**Fig. 9.13:** Production of monoclonal antibodies, (HAT, hypoxanthine aminopterin and thymidine; HGPT, hypoxanthine-guanine phosphoribosyltransferase).

2. The spleen of the mouse is removed and a suspension is made. The suspension includes B cells that produce antibodies against the injected antigen.
3. The spleen cells are then mixed with myeloma cells that are capable, but have lost the ability to produce antibodies. Some of the antibody-producing spleen

cells and myeloma cells fuse to form hybrid cells. These hybrid cells are now capable of growing continuously in culture, while producing antibodies.

4. The mixture of cells are placed in a selective medium (HAT medium) that allows only hybrid cells to grow.
5. Hybrid cells proliferate into clones called hybridomas. The hybridomas are screened for production of the desired antibody.
6. The selected hybridomas are then cultured to produce large amounts of monoclonal antibodies.
7. The hybridomas can be maintained *in vivo* in mouse and stored *in vitro* in freeze-dried form.

The discovery of hybridoma technology for production of monoclonal antibodies, has created a revolution in immunology by opening up numerous diagnostic, therapeutic and research applications.

Monoclonal antibodies are being employed in many areas, where antisera were formerly used, like blood-grouping, tissue typing and hormone radioimmunoassay. Several diagnostic procedures that use monoclonal antibodies are available. Generally, these procedures are quicker and more accurate than previously used procedure. For example, a monoclonal antibody can be used to detect pregnancy only 10 days after conception.

Monoclonal antibodies allow rapid diagnosis of hepatitis, influenza, herpes, streptococcal and chlamydial infections. Monoclonal antibodies can be used to analyze complex biological systems ranging from molecules, on the surface, to the organization of whole tissues.

Because of its exquisite binding specificity, monoclonal antibodies can be used to isolate particular substance from a mixture in which it may be present in a very small quantity (e.g. interferon).

They are also being explored as tools of clinical imaging and therapy in cancer. For example, certain radioactively-labeled monoclonal antibodies that recognize tumor surface antigen, if injected into the bloodstream, home to the tumor and reveal its location by radioactive emission. Similarly, monoclonal antibodies coupled to toxin such as ricin and diphtheria toxin (immunotoxin) and also cytotoxic drugs have shown some promise as, antitumor chemotherapeutic agents capable of targeting toxic activity and drugs, specially, on tumor cells. Reactivity of monoclonal antibodies on leukemic cells and their ability to kill is under study.

The therapeutic use of monoclonal antibodies has been limited, because these antibodies are currently produced by mouse cells. The immune systems of some people have reacted against the foreign mouse proteins. Monoclonal antibodies derived from human cells would probably provoke fewer reactions. Several approaches are being followed to solve this problem. One is to construct, by recombinant deoxyribonucleic acid (DNA) technology, antibody with variable regions derived from mouse cells and constant regions derived from human sources. These antibodies are more compatible to human system, are called chimeric monoclonal antibodies. Genetic engineering also can be used to alter mouse antibodies, so that they have characteristics that are more common.

## Factors Influencing Antibody Production

### *Genetic Factors*

Different individuals show difference in their ability to resist infectious diseases and allergic diseases and these differences are determined genetically and are being controlled by immune response (Ir) gene, which is situated in the short arm of the sixth chromosome. MHC class II genes are referred to as Ir gene. The Ir genes determine the magnitude of immune response to a particular antigen, whether it is a pathogen or an allergen.

### *Age*

The embryo is immunologically immature. The immunological competence is achieved after the development of lymphoid organs. When the potential immunocompetent cells during embryonic life, comes in contact with specific antigen, the response is elimination of cells (clonal deletion) or induction of tolerance. This is believed to be the basis of non-antigenicity to self-antigen.

At birth also, the infant is not immunologically competent, because it has to depend on antibody present in mother's milk. Full competence is achieved only by about the age of 4 years.

### *Nutritional Status*

Malnutrition affects immune response, both antibody-mediated immunity (AMI) and cell-mediated immunity (CMI), adversely. Deficiency of amino acids (tryptophan phenylalanine, methionine, glycine and isoleucine) and vitamins (A and B group) have been shown to decrease the antibody production.

### *Route of Administration*

Whether, a substance will evoke an immune response also depends on the route by which it enters the body. A quantity of substance that has no effect when injected intravenously, may evoke a copious antibody response, when injected subcutaneously. Route of contact also can influence the type of antibody produced. Oral and nasal route of administration may cause secretory IgA production, where as inhalation of pollen antigen induces IgE synthesis. Application of simple chemicals to the skin may lead to cellular immune response.

### *Size and Number of Doses*

The threshold dose required for a response, under particular conditions varies among immunogens. When the dose is increased, the response increases. This happens up to a critical level beyond which the excessive doses may not only fail to induce response, but may instead establish a state of specific unresponsiveness or tolerance to the subsequent exposures to that antigen, is sometimes referred to as high-zone tolerance. Massive antigenic load, at times, appears to swamp the antibody-producing system and paralyse it. This phenomenon is known as immunological paralysis.

The increased antibody response to an antigenic stimulus, subsequently, has been noticed (secondary response). With repeated antigen injections, the antibody response increase progressively, up to a certain extent after which there will be no increase.

### *Multiple Antigens*

When two or more antigens are given simultaneously, the effects may vary. When two bacterial vaccines (cholera and typhoid) are given together in a mixed form, the antibody response is not influenced by the other. But, when toxoids are given along with killed bacteria, the actions of toxoids are potentiated (triple vaccine). When toxoids (diphtheria and tetanus) are given together and the dose of one is in excess, it will inhibit the action of other. Therefore, for maximum effect, the nature and relative proportion of different antigens in a mixture should be carefully adjusted.

### *Adjuvants*

The response to an immunogen can be enhanced, if it is administered as a mixture with substance called adjuvants. Adjuvants function in several ways:

1. Increase the immunogenicity of non-antigenic substances.

2. Due to its slow release, it increases the concentration and persistence of circulating antibody.
3. Increase the size of the immunogen, hence facilitate phagocytosis and antigen presentation by the macrophages.
4. Induce and enhance cell-mediated immunity.
5. Promote local cytokine production and other immunologic activities of such immune cells.

Examples are:

1. Aluminium salts (both phosphate and hydroxide).
2. Freund's incomplete adjuvant (protein antigen incorporated in water phase of water in oil emulsion).
3. Freund's complete adjuvant (incomplete adjuvant along with suspension of killed tubercle bacilli).
4. Others such as silica particles, beryllium sulfate, endotoxins, etc.

The adjuvants delay the release of antigen from the site of injection and prolong the antigenic stimulus.

Another promising approach is to incorporate specific immunomodulators into the vaccine, to promote particular type of immune response. IL-12 influences $T_h$ cell activity to promote cell-mediated reaction, particularly promoting cytotoxic response against target pathogens.

### *Immunosuppressive Agents*

Immunosuppressive agents inhibit the immune response. The commonly employed are:

1. X-irradiation.
2. Radiomimetic drugs.
3. Corticosteroids.
4. Antimetabolites.
5. Antilymphocytic serum (ALS).

Sublethal dose of irradiation suppresses antibody formation. 24 hours after irradiation, there will be no antibody formation.

Alkylating agents (cyclophosphamide, nitrogen mustard, etc.) suppress antibody formation. Corticosteroids cause depletion of lymphocytes from blood and lymphoid tissue. Corticosteroids given in therapeutic doses for a short period will have little effect on antibody production. Corticosteroids suppress delayed hypersensitivity.

Antimetabolites such as methotrexate (folic acid antagonist), 6-mercaptopurine (analogues of purine), etc. inhibit the DNA and ribonucleic acid (RNA) synthesis, inhibiting the cell division and differentiation of cells necessary for humoral and cell-mediated immune response.

Antilymphocytic serum is a heterogeneous antiserum raised against T lymphocytes or thymocytes. ALS is indicated in transplanted surgery suppressing the cell-mediate immunity (CMI), thus preventing graft rejection.

### *Effect of Antibody*

Passively administered IgG, suppresses the homologous antibody synthesis by a feedback process. The antibody may also combine with the antigen and prevents its availability for immune-competent cells. This property has been utilized in the isoimmunization of women by the administration of anti-Rh (D) IgG during delivery.

Intravenous administration of immunoglobulin has been shown to have immunomodulatory effects. It is being used in the treatment of many immunopathologic disorders such as thrombocytopenia, autoimmune hemolytic anemia, etc.

## CELL-MEDIATED IMMUNE RESPONSE (CELL-MEDIATED IMMUNITY)

Humoral immunity, which involves B cells, $T_h2$ cell and antibodies is efficient in removing pathogens and foreign substances from the body fluid. However, in case of

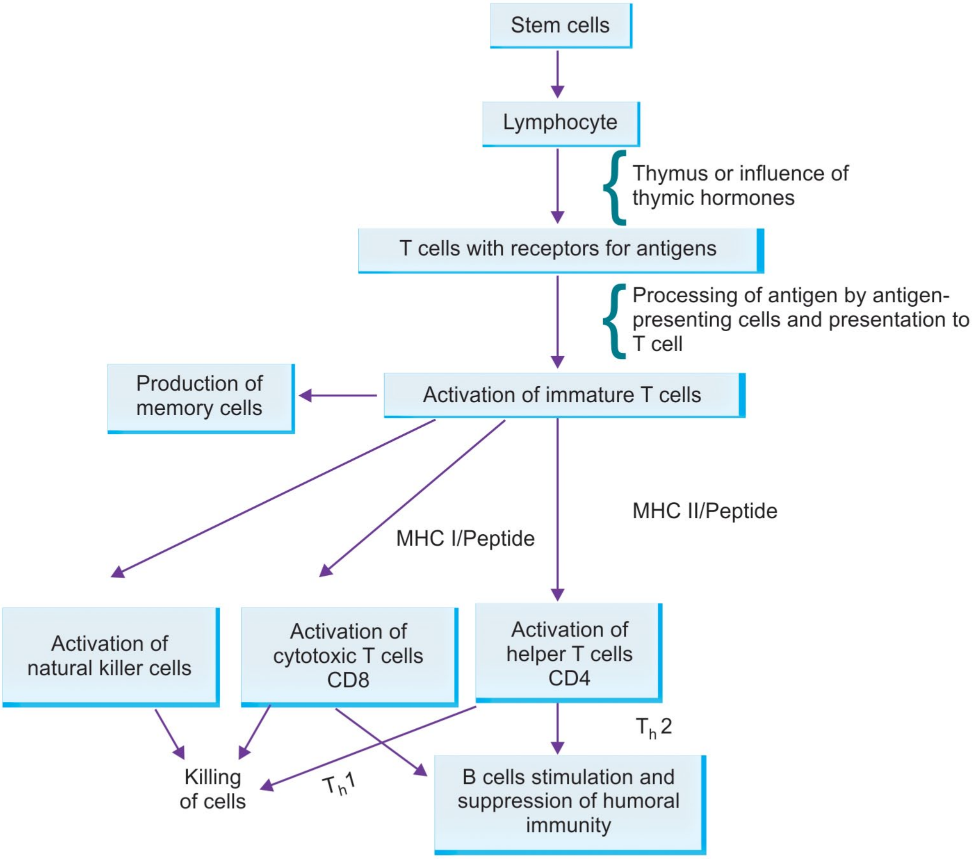

Stem cells
Lymphocyte
Thymus or influence of thymic hormones
T cells with receptors for antigens
Processing of antigen by antigen-presenting cells and presentation to T cell
Production of memory cells
Activation of immature T cells
MHC I/Peptide
MHC II/Peptide
Activation of natural killer cells
Activation of cytotoxic T cells CD8
Activation of helper T cells CD4
T_h2
Killing of cells
T_h1
B cells stimulation and suppression of humoral immunity

**Fig. 9.14:** Summary of cell-mediated immunity

intracellular pathogens, antibodies are ineffective, because of their inaccessibility to the antigen. The cell-mediated immune response, then, comes to play. This is a specific type of immune response, which does not involve antibodies (Fig. 9.14).

## Scope of Cell-mediated Immunity

Cell-mediated immunity serves following immunological functions:

- Immunity against obligate intracellular bacteria *(Mycobacterium tuberculosis, M. leprae, Listeria monocytogenes, Brucella,* etc.), fungi *(Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis,* etc), protozoa *(Leishmania)* and malaria species) and viruses (smallpox, measles, mumps, etc.)
- Participation in the mechanism and pathogenesis of delayed hypersensitivity reaction
- T cell-mediated immune response accounts for rejection of transplants
- Immunological surveillance and immunity against cancer
- Pathogenesis of certain autoimmune diseases (e.g. thyroiditis, encephalitis, etc).

T cells, as noted earlier, mature in thymus and develop under the influence of thymic hormones, unlike B cells, they do not make antibodies. However, they do have surface membrane receptors that can recognize peptide fragments that are bound to MHC molecules. The cell-mediated immune response involves the differentiation and the actions of different type of T cells and the production of chemical mediators or cytokines.

## Cell-mediated Immune Reactions

Cell-mediated immune reactions begin with processing of the antigen by APCs described earlier. Amongst the APCs, dendritic cells are found in lymphoid tissues, where T cells are located. Antigen processing involves the ingestion and degradation of pathogen within an APC. However, during degradation process, some antigen fragments (peptides) are associated with MHC molecules either MHC I, if antigen is endogenous mostly or MHC II in case of exogenous antigen. These MHC-peptide complexes are then presented on the membrane surface of the APC.

When naive T cells face antigenic challenge, the cells mature into one of the several types of T cells. During differentiation, some T memory cells are formed. As in humoral immunity, the persistence of memory cells in CMI allows the body, to recognize antigens to which T cells have previously reacted and to mount more rapid subsequent responses. As it does for B cells, clonal deletion in the thymus removes those T cells that bear receptors for self-antigens.

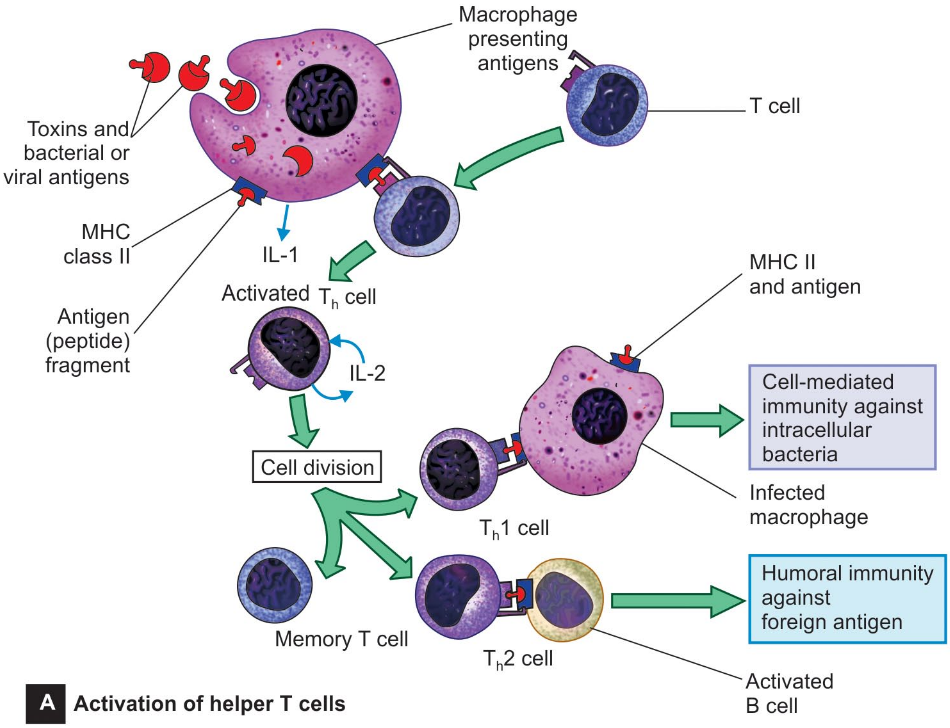
### *T Cell Involvement*

When a macrophage or dendritic cell presents MHC class II-peptide complex on its surface, only T cells with proper receptor bind to the complex. Binding stimulates macrophages and the T cells to secrete IL-1 and IL-2 respectively. IL-2 helps the T cells to divide and differentiate into activated T helper cells. Most of these mature $T_h$ effector cells belong to one of the two distinct subsets designated as $T_h1$ and $T_h2$ cells, that are distinguished by the particular lymphokines they produce (Table 9.1). Their divergent patterns of lymphokines expression, in turn, allows each of these $T_h$ subsets to promote distinct types of immune reactions that are best suited to eliminating particular types of microorganisms. The cytokines produced by each of the two $T_h$ subsets reciprocally inhibit the development of others. Thus, $T_h1$ derived IFN-γ inhibits the development of $T_h2$ cells and IL-4 produced by $T_h2$ prevents development of $T_h1$ (Figs 9.15A and B).

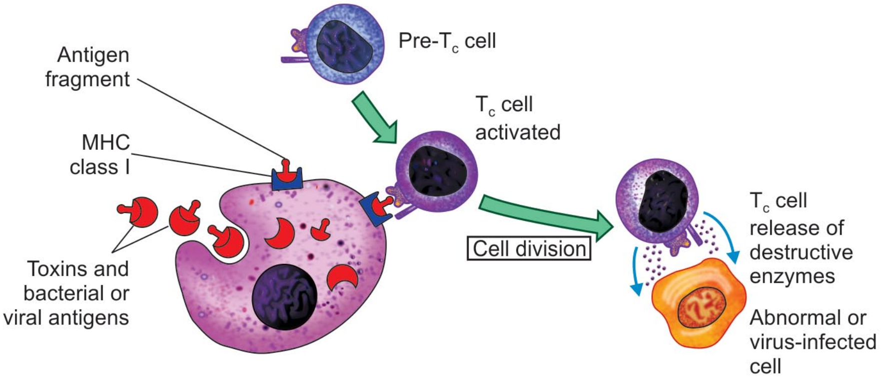
### *Differentiation of Helper T Cells to $T_h1$ or $T_h2$*

Both $T_h1$ and $T_h2$ cells are derived from a common precursor, i.e. $T_hO$ cell, which can differentiate to $T_h1$ or $T_h2$ (Fig. 9.16). Cytokines are the major determinants of such diversification. IL-12, secreted by activated macrophage is the main cytokine that leads to differentiation of naive T helper cells to $T_h1$. On the other hand, IL-4 is the main cytokine responsible for differentiation to $T_h2$ cells (Fig. 9.17).

*$T_h1$ cells:* These cells produce IL-2, IFN-γ and TNF-β. Some bacteria such as *M. tuberculosis, M. leprae, Brucella* species, some fungi (*Pneumocystis carinii* causing *Pneumocystis* pneumonia, etc.) can grow in cytoplasmic vesicles, even after they are engulfed by releasing interferon γ. IFN-γ potentially activates macrophages by inducing nitric oxide synthetase and other metabolic enzymes that increase microbicidal activity. At the same time, IFN-γ acts on activated B cells, to induce immunoglobulin switching to IgG-1, an isotype that binds strongly to all three classes of macrophages Fc receptors and so function as an extremely potent opsonin. The overall effect is to potentiate both engulfment and killing by phagocytosis.


Macrophage presenting antigens
T cell
Toxins and bacterial or viral antigens
MHC class II
IL-1
Activated $T_h$ cell
Antigen (peptide) fragment
IL-2
MHC II and antigen
Cell-mediated immunity against intracellular bacteria
Cell division
Infected macrophage
$T_h$1 cell
Humoral immunity against foreign antigen
Memory T cell
$T_h$2 cell
Activated B cell

**A** **Activation of helper T cells**


Pre-$T_c$ cell
Antigen fragment
$T_c$ cell activated
MHC class I
$T_c$ cell release of destructive enzymes
Cell division
Toxins and bacterial or viral antigens
Abnormal or virus-infected cell

**B** **Activation of cytotoxic T cells**

**Figs 9.15A and B**: The reactions in cell-mediated immunity. **A.** The macrophage has processed an antigen and inserted an antigen (peptide) fragment into its plasma membrane as a major histocompatibility complex (MHC) class II molecule. $T_h$ cells have receptors that recognize the peptide fragment on MHC class II. Binding causes the $T_h$ cells to become activated. The activated $T_h$ cells then differentiate into either $T_h$1 cells or $T_h$2 cells. $T_h$1 cells activate infected macrophages to destroy internal bacterial infections. $T_h$2 cells activate B cells (humoral immune responses) by binding to MHC class II: peptide presented by the B cells; **B.** Presenting the same peptide fragment on MHC class I to $T_c$ cells activate these cells to attack infected cells, especially abnormal or virus-infected cells.

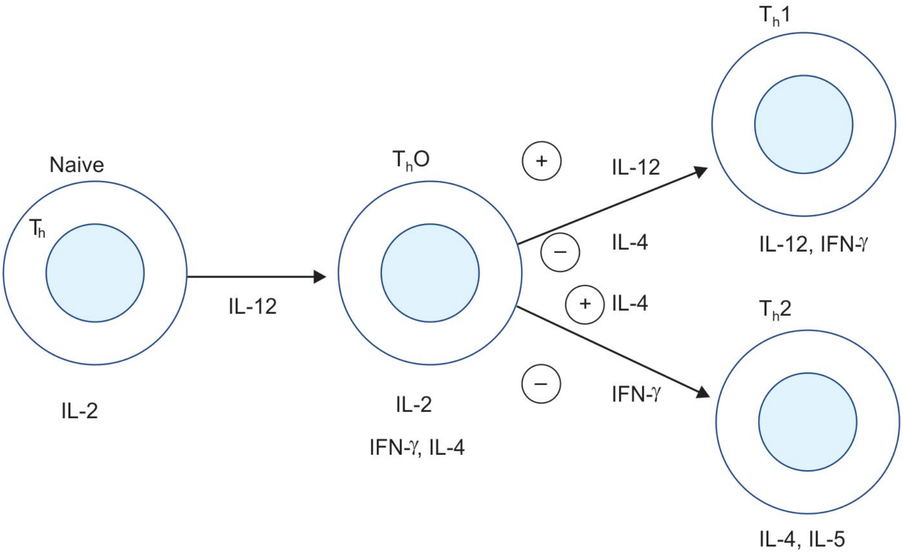


**Fig. 9.16:** Differentiation of helper T cells into $T_h1$ and $T_h2$ cells. Naive $T_h$ cells produce IL-2 and little amount of other cytokines on initial activation. Repeated stimulation in the presence of IL-12, a macrophage-derived cytokine, causes $T_h$ cells to differentiate into $T_h1$ cells, which produce IL-2 and IFN-γ and are particularly effective in enhancing immune responses that involve macrophages and other phagocytes. Stimulation in the presence of IL-4, on the other hand, promotes the development of $T_h$ cells, which produce IL-4 and other cytokines that promote mast cell and eosinophil-mediated responses. The differentiation into either $T_h$ subtype probably involves a common intermediary, designated $T_hO$, which produces IL-2, INF-γ and IL-4. $T_h1$ and $T_h2$ cells have the ability to mutually down-regulate the development of the other: the $T_h1$ product IFN-γ impairs the generation of $T_h2$ cells and the $T_h2$ cytokine IL-4 inhibits the development of $T_h1$ cells.

*$T_h2$ cells:* These cells do not produce IFN-γ, IL-2 and TNF-β like $T_h1$ cells. On the other hand, they secrete IL-4, IL-5, IL-6, IL-10 and IL-13. These $T_h2$ derived cytokines act together to chemotract B cells, mast cells, basophils and eosinophils and promote growth and differentiation of cells at the immune response site. In addition, IL-4 helps the B cells for class switching to IgE, the isotype, which bound uniquely to the Fc-epsilon receptors present on the mast cells and eosinophils, which enable those cells, recognize and react to antigens.

This type of defense mechanism mostly occurs against large multicellular parasites (helminths such as *Schistosoma*), which cannot be killed by phagocytosis. In $T_h2$-mediated response, macrophage plays no role. This is because firstly, IL-10 inhibits INF-γ on which macrophage activation depends. Secondly, IL-4 selectively favors the production of two immunoglobulin isotopes (IgE and IgG4) that are not recognized by macrophage Fc receptors.

### *Cytotoxic T Cells*

The $T_c$ cells recognize the antigenic peptides (virus-infected cells, tumor cells) in association with MHC I molecule refer (Refer Fig. 9.15B). The peptide-MHC I complex is the first signal for the activation of $T_c$ cells. The second signal is offered by IL-2 secreted

**Table 9.1** Lymphoke expression by Th1 and Th2 cells

| Th subtype | Cytokines secreted | Major immunologic effects |
|---|---|---|
| $T_h1$ | IFN-γ* | Activate macrophages promote B cell proliferation and class switching to IgG1 |
| | IL-2† | Promotion activation of antigen specific $T_h1$ and $T_c$ cells |
| | TNF-β‡ | Activate macrophages and neutrophils. Promote B cell growth and immunoglobulin production |
| $T_h2$ | IL-4 | Chemoattractants lymphocytes, mast cells and basophils. Enhance growth of mast cells and eosinophils<br>Promote B cell proliferation and class switching to IgE and IgG4 inhibit $T_h1$ cell differentiation. Inhibit cytokine production by macrophages |
| | IL-5 | Enhance growth and development of eosinophils |
| | IL-6 | Promote B cell growth and immunoglobulin production |
| | IL-10 | Inhibit production of cytokines (including IFN-γ) by $T_h1$ cells, macrophages and other APCs§<br>Inhibit $T_h1$ cell growth and immunoglobulin production |
| | IL-13 | Same as IL-4 |

*IFN-γ, interferon gamma; †IL-2, interleukin-2; ‡TNF-β, tumor necrosis factor beta; §APCs, antigen-presenting cells.

by near $T_h$ cells. Following these two signals, activation of $T_c$ cells lead to the destruction of virus-infected cells, tumor cells or foreign cells (in the form of transplant).

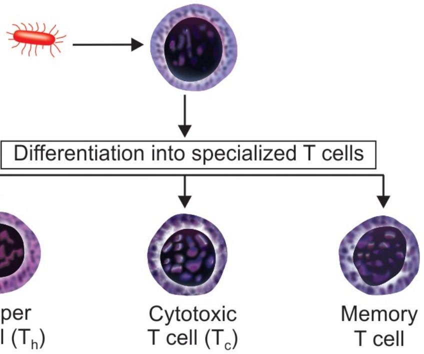


**Fig. 9.17:** After T cells are challenged by antigens, the cells differentiate into one of the several types of functioning T cells.

## CYTOKINES

Cytokines are soluble mediators, glycoprotein in nature, produced by and act on various immune and non-immune cells. They are the intercellular messengers not only regulate immune and inflammatory response, but also wound healing, hematopoiesis, angiogenesis and many other biological processes. The cytokines permit them to regulate cellular activity in a coordinated and interactive fashion. Cytokines exhibit the properties of pleiotropy, redundancy, synergy, antagonism and cascade induction. A cytokine is said to be pleiotropic, when it has different biological effects on different target cells. When two or more cytokines mediate similar function they are said to be redundant; redundancy makes it difficult to describe a particular activity to a single cytokine. When the

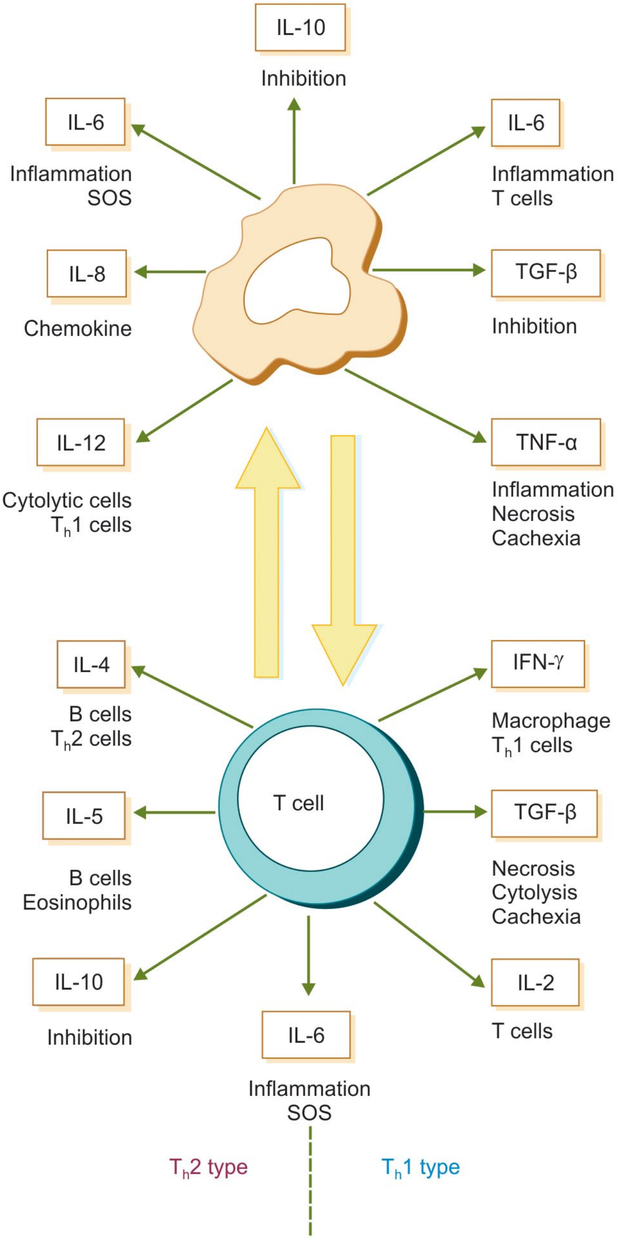

IL-10
Inhibition
IL-6
Inflammation
SOS
IL-6
Inflammation
T cells
IL-8
Chemokine
TGF-β
Inhibition
IL-12
Cytolytic cells
$T_h1$ cells
TNF-α
Inflammation
Necrosis
Cachexia
IL-4
B cells
$T_h2$ cells
IFN-γ
Macrophage
$T_h1$ cells
T cell
IL-5
B cells
Eosinophils
TGF-β
Necrosis
Cytolysis
Cachexia
IL-10
Inhibition
IL-6
Inflammation
SOS
IL-2
T cells
$T_h2$ type
$T_h1$ type

**Fig. 9.18:** Major cytokines. This figure shows the cytokines of major relevance to immunity against intracellular bacteria. Monocyte or T cell-derived cytokines are often termed monokines or lymphokines, respectively. Cytokines of $T_h1$ type are central to protective immunity against intracellular bacteria, while cytokines of $T_h2$ type are important for protection against helminths and in allergic responses.

combined effect of two cytokines is greater than the adaptive effect of individual cytokine, it is referred to as cytokine synergism. On the other hand, when the effect of one cytokine is inhibited by another cytokine, it is called cytokine antagonism. Cascade induction occurs, when action of cytokine on the target cell induces production of cytokines, which in turn may induce other target cells to produce other cytokines.

The outcome of particular response does not depend upon a single cytokine, instead, on the cytokine milieu. The cytokines of major relevance to immunity against intracellular bacteria are described (Fig. 9.18).

## Features of Some Important Cytokines

### *Interleukins*

Many cytokines are referred as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins (1–25) have been identified. Some interleukins and their functions are listed in Table 9.2.

*Interleukin-1 (IL-1):* It was previously known as endogenous pyrogen, lymphocyte-activating factor (LAF). IL-1 is produced by many cells such as macrophages, endothelial cells, B cells, fibroblast, but macrophages produce IL-1 abundantly. It stimulates T and B cells and induces inflammatory responses. IL-1 together with TNF-α goes to brain, where they induces fever and act to increase corticosteroid release. In the liver, it induces production of acute phase proteins. It mediates a wide range of metabolic, physiological inflammatory and hematological effects by acting on bone marrow, epithelial cells, fibroblasts, osteoclasts, hepatic cells, etc. Virtually, all cells of the body have receptors for IL-1 and can respond to it (Fig. 9.19).

*Interleukin-2 (IL-2):* Previously, it was known as T-cell growth factor (TCGF). IL-2 is chiefly produced by $T_h1$ cell of $CD4^+$ series and also by $CD8^+$ cells. It has action on restricted range of cells, chiefly on T cells. It also acts on NK cells and B cells. It transforms some null cells—large granular lymphocytes (LGL) to lymphokine-activated killer cells (LAK cells), which can kill cancer cells (Fig. 9.20).

*Interleukin-3 (IL-3):* It is called multi-colony stimulating factor (CSF), as it stimulates the growth of precursors of all the hematopoietic lineage cells.

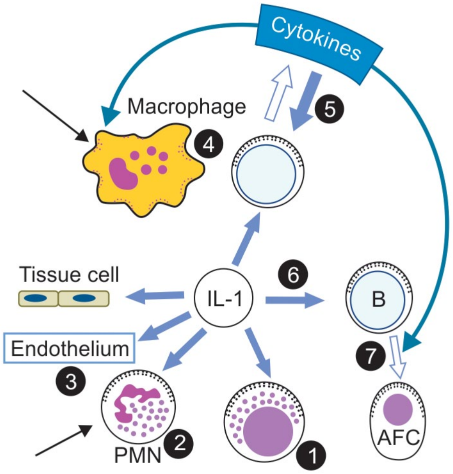


**Fig. 9.19:** Interleukin-1 is produced by many cell types in response to damage, infection or antigens. It influences many cells and processes. **1.** NK cell—cytocidal activity increases; **2.** Polymorphonuclear neutrophils (PMNs) are metabolically activated and move towards the site of IL-1 production by chemotaxis (black arrow); **3.** In the endothelium, adhesion molecules and procoagulants are induced and permeability is increased; **4.** Prostaglandin production and cytocidal activity increase in macrophages. Chemotaxis is also stimulated (black arrow); **5.** $T_h$ cell proliferation, IL-2 receptor expression and cytokine production are all enhanced; **6.** B cell proliferation and differentiation into antibody-forming cells (AFCs) is stimulated and regulated; **7.** By other cytokines.

**Table 9.2** Major properties of human interleukins

| Interleukin | Principal cell source | Principal effects |
|---|---|---|
| IL-1[*] | Macrophages, other APCs[†], other somatic cells | Costimulation of APCs and T cells; B cell growth and Ig production acute phase response. Phagocytic activation, inflammation and fever; promotes hematopoiesis |
| IL-2 (TCGF)[‡] | Activated $T_h$1 cells NK[§] cells CTL[‖]. Proliferation of activated T cells | Proliferation of activated T cells; apoptosis of T cells after prolonged or repeated activation; NK cell and CTL function; B cell proliferation and IgG2 expression |
| IL-3 (multi-CSF[¶]) | T lymphocytes | Growth of early hematopoietic progenitors.<br>B cell proliferation, IgE |
| IL-4 | $T_h$2 cells, mast cells | B cell proliferation, IgE expression, class II MHC[**] expression, $T_h$2 cell and CTL proliferation and eosinophil and mast cell growth function<br>Inhibit monokine production |
| IL-5 | $T_h$2 cells, mast cells | Eosinophil growth and function |
| IL-6 | Activated $T_h$2 cells, APCs, other somatic cells | Synergistic effect with IL-1 or TNF[††], fever, acute phase response, B cell growth and Ig production, hematopoiesis |
| IL-7 | Thymic and marrow stromal cells | T and B lymphopoiesis; CTL functions |
| IL-8 | Macrophages, other somatic cells | Chemoattractants neutrophils and T cells |
| IL-9 | T cells | Hemopoietic and thymopoietic effects |
| IL-10 | Activated $T_h$2, CD8[‡‡] T and B lymphocyte, macrophages | Inhibit cytokine production by $T_h$1 cells, NK cells and APCs promote B cell proliferation and antibody responses. Suppresses cell-mediated immunity |
| IL-11 | Stromal cells | Synergistic effect on hemopoiesis and thrombopoiesis |
| IL-12 | B cells, macrophages | Proliferation and function of activated CTLs and NK cells; IFN[§§]-γ production. Promotes $T_h$1 and suppress $T_h$2 functions; promotes CM9 |
| IL-13 | $T_h$2 cells | Similar to IL-4 effects |
| IL-15 | Epithelial cells and monocytes, non-lymphocytic cells | Mimic IL-2 T cell effect mast cell and NK activation |
| IL-16 | CD8 and some CD4 T lymphocytes | Chemoattractants CD4 T cells, eosinophils and monocytes |
| IL-17 | Activated memory T cells | Promotes T cell proliferation, neutrophil development |
| IL-18 | Macrophages, keratinocytes | Coinduces IFN-γ production, coactivates $T_h$1 and NK cell development |

[*] IL-1, interleukin-1; [†] APCs, antigen-presenting cells; [‡] TCGF, T-cell growth factor; [§] NK, natural killer cell; [‖] CTL, cytotoxic T lymphocyte; [¶] CSF, colony stimulating factor; [**] MHC, major histocompatibility complex; [††] TNF, tumor necrosis factor; [‡‡] CD8, cluster of differentiation 8; [§§] IFN, interferon.

*Interleukin-4 (IL-4):* Earlier it was called B cell-activator or differentiating factor. It is secreted by activated T cells ($T_h2$). It acts on B cell to induce differentiation to produce IgG1 and IgE. It also acts on T cells as a growth and activation factor for $T_h2$ differentiation. IL-4 is secreted by $T_h2$ cells, mast cells and a subset of NK cells. IL-4 is now best known for the role it plays in allergic diseases by promoting IgE production.

*Interleukin-5 (IL-5):* Originally, it was described as a B cell growth factor (BCGF) in mice, but functions mainly as an eosinophil growth and differentiation factor in humans. $T_h2$ cells are the main source of IL-5.

*Interleukin-6 (IL-6):* Earlier it was known as B-cell differentiation factor (BCDF) or hepatocyte stimulating factor. IL-6 produced by many cells including T cells, macrophages B cells fibroblasts and endothelial cells.

In addition to enhancing B-cell replication, differentiation and immunoglobulin production, the major activities of IL-6 include synergizing with IL-1 and TNF to promote T cell activation and also induce acute phase response.

*Interleukin-7 (IL-7):* It is secreted by the stromal cells of bone marrow, thymus and spleen. It gives proper signal to the precursors of both B and T cells. IL-7 increases macrophage cytotoxic activity and induces cytokine secretion by monocytes.

*Interleukin-8 (IL-8):* It is a powerful inducer of neutrophil chemotaxis. IL-8 is produced by most cells of the body including macrophages and endothelial cells and are associated with inflammation and cell migration.

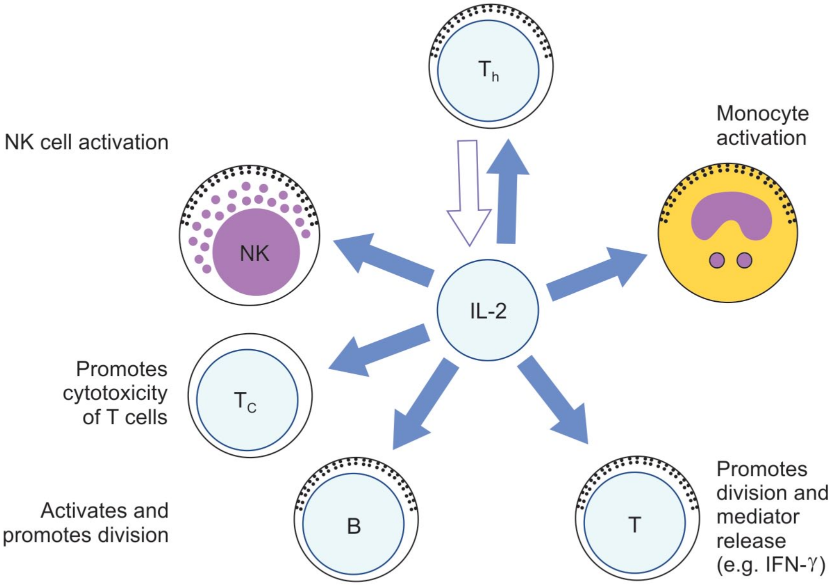

T<sub>h</sub>
Monocyte activation
NK cell activation
NK
IL-2
Promotes cytotoxicity of T cells
Tc
Activates and promotes division
B
T
Promotes division and mediator release (e.g. IFN-γ)

**Fig. 9.20:** Interleukin-2 is generated by $T_h$ cells. In addition to the essential role in promoting T cell division and the release of mediators such as IFN-γ, IL-2 also potentiates B cell growth. The activation of monocytes and natural killer (NK) cells is important in amplifying the immune response. In patients with renal cell carcinoma, autologous NK precursors can be activated *in vitro* by high doses of IL-2 (1,000 IU/mL) to produce lines of so called lymphokine-activated killer (LAK) cells, which are used in experimental cancer therapy.

*Interleukin-9 (IL-9):* It is secreted by activated T cells and is a promoter of T cells and mast cells. It synergizes with IL-2 or IL-4 in T cell costimulation and may also stimulate hematopoietic progenitors. Its physiologic role is not established.

*Interleukin-10 (IL-10):* It is a product of activated $T_h2$ and $CD8^+$ cells, B cells monocytes and keratinocytes. It is an inhibitor of immune response being responsible for suppressing class II MHC expression on macrophages and dendritic cells. IL-10 inhibits the production of IL-2 and IFN by $T_h1$ cells, there by favoring $T_h2$-dependent responses. The production of IL-1, TNF-α and IL-6 by macrophages are diminished, because of the defect in antigen presentation.

*Interleukin-11 (IL-11):* It is an important growth factor for thrombocytes. Stromal cells are the principal source of IL-11. IL-11 has synergistic effects on hemotopoiesis and thrombopoiesis. IL-11 can induce production of some acute phase proteins (APP) as IL-6.

*Interleukin-12 (IL-12):* It is produced by macrophages, dendritic cells and activated B cells. It is a critical regulator of both in nate and acquired immunity. It potentiates CMI by promoting differentiation of $T_h1$ cells. Simultaneously, it suppresses $T_h2$ cell response leading to less production of IL-4, IL-10 and IgE antibodies. It enhances proliferation of activated T and NK cells, thus most potent inducer of IFN-γ.

*Interleukin-13 (IL-13):* It has structural and functional similarities with IL-4.

*Interleukin-15 (IL-15):* Many biological properties of IL-15 are similar to that of IL-2, thus enhances proliferation of activated T cells, generation of cytotoxic T cells and LAK cells. It is also essential for the development and survival of NK cells. It is reported that it promotes $T_h1$ cell response preferentially.

*Interleukin-16 (IL-16):* It is produced by CD8 cells and acts as a chemoattractant for CD4 cells. IL-16 does prevent human immunodeficiency virus (HIV) replication by blocking viral messenger (RNA) expression.

*Interleukin-17 (IL-17):* It is produced by activated memory T cells and binds to the receptors on many cells, particularly on cells of the spleen and kidney. IL-17 induces the target cells to express IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF). It stimulates neutrophil precursor cells.

*Interleukin-18 (IL-18):* It is produced by keratinocytes and macrophages. It is structurally related to IL-1. It potentiates IFN-γ and GM-CSF production by T, B and NK cell and promotes $T_h2$ differentiation.

### *Interferons (Antiviral Cytokines)*

Interferon consists of a large family of secretory proteins that not only share antiviral activity, but also have the ability to inhibit the growth of cells and to modulate immune responses. There are three classes of interferons: IFN-α, IFN-β and IFN-γ. Both IFN-α and IFN-β are produced by leukocytes and fibroblasts respectively and have cell growth inhibition and antiviral property. The IFN-γ is produced by activated T cells and NK cells. It is an immune modulator through weakly antiviral. IFN-γ induces transformation of ordinary macrophage to activated macrophage, to deal better against the intracellular bacterial infection. Further, it increases APC function by inducing MHC class II molecules. The properties of IFN-γ are summarized (Fig. 9.21).

Excessive production of IFN-γ can play a part in the induction of autoimmunity.

## Biological Functions of Cytokines

Cytokines are involved in a wide range of biological activities including innate immunity, adaptive immunity, inflammation, wound healing and hematopoiesis. Presently, the number of proteins with cytokine activity exceeds 100. As the time passes, new ones are

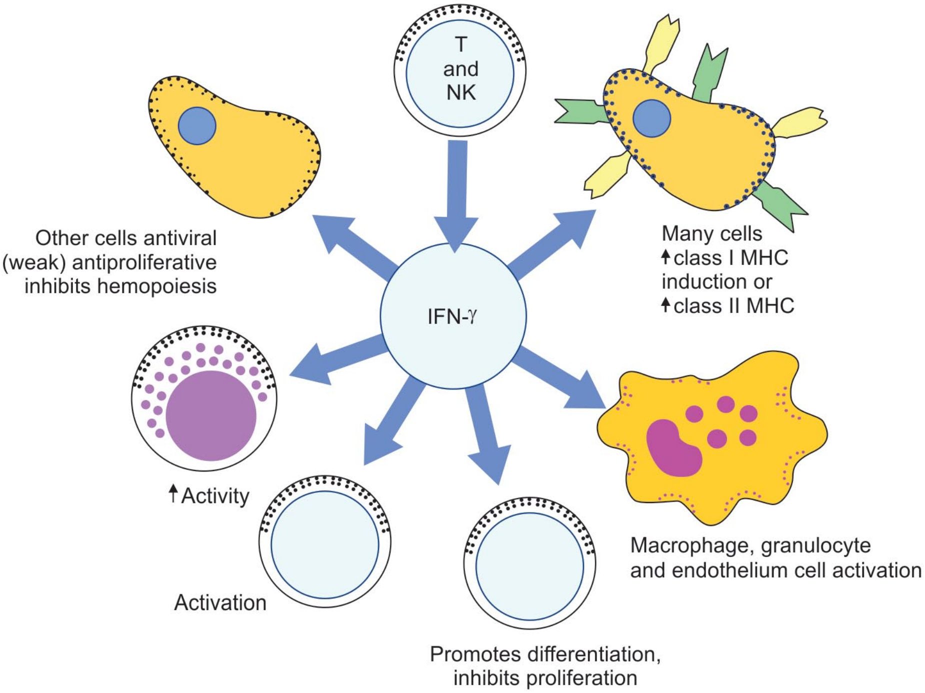

T and NK
IFN-γ
Other cells antiviral (weak) antiproliferative inhibits hemopoiesis
Many cells ↑class I MHC induction or ↑class II MHC
↑Activity
Activation
Promotes differentiation, inhibits proliferation
Macrophage, granulocyte and endothelium cell activation

**Fig. 9.21:** IFN-γ has numerous immunoregulatory actions. Its antiviral and antiproliferative activities are less potent than those of IFN-α and IFN-β. Furthermore, it is not as effective as IFN-α at inducing natural killer (NK) cells. It is however, the most potent inducer of macrophage activation and of class II molecules on tissue cells. In this and other functions it synergizes with TNF-α and TNF-β.

added to the list. Unlike antibody, which acts specifically with its antigen, the cytokines act in an antigen non-specific manner. That is they affect, whatever cells they encounter that bears appropriate receptors and are in a physiological state that allows them to respond.

## Cytokine Receptors

In order to act on the target cells and exert their biological effects, cytokines must bind to the specific receptors expressed on the membrane of the responsive target cells. Cloning of the genes encoding cytokine receptors has contributed to rapid advances for identification and characterization of these receptors.

Cytokines receptors are classified under five families of receptor proteins as follows (Fig. 9.22):

1. Immunoglobulin superfamily receptor (IL-1, M-CSF and kit).
2. Class I cytokine receptor family (hematopoietin receptor family). They include IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, GM-CSF, G-CSF, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), growth hormone prolactin (Table 9.3).
3. Class II cytokine receptor family (interferon receptor family). They include IFN-α, IFN-β, IFN-γ, IL-10.

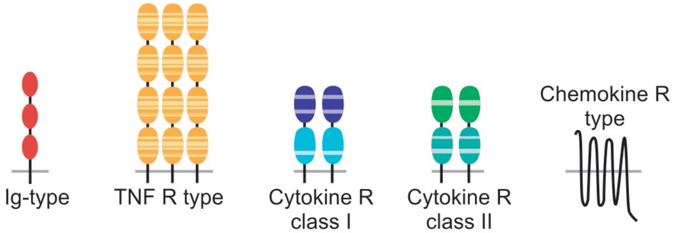


**Fig. 9.22:** Schematic diagrams showing the structural features that define the five types of receptor proteins to which most cytokines bind. The receptors for most of the interleukins belong to the class I cytokine receptor family. C refers to conserved cysteine.

4. Tumor necrosis factor receptor family (TNF-α, TNF-β, CD40, nerve growth factor, FAS).
5. Chemokine receptor family.

## Cytokine Antagonists

There are certain proteins, which inhibit the biological activity of cytokines. The action of cytokines may be blocked in two ways, one by blocking the cytokine receptor and the other by blocking the circulating cytokines. The best characterized receptor is IL-1 receptor-antagonist (IL-1 Ra), which binds IL-1R by blocking the action of IL-1 cytokine on IL-1R.

Cytokine inhibitors are also found in the blood and extracellular fluid. These soluble antagonists arise from the enzymatic cleavage of the extracellular domain of the cytokine receptors. These soluble receptors include (IL-2, IL-4, IL-6, IL-7, IFN-γ, IFN-α, TNF-β, etc).

Some viruses produce cytokine-like substances, which mimic cytokines. For example, Epstein-Barr virus (EBV), an IL-10-like molecule (viral IL-10 or vIL-10) that binds to IL-10 receptors and like cellular IL-10, suppresses $T_h1$ type cell-mediated responses. The molecules produced by viruses, that mimic cytokines, allow the virus to manipulate the immune response, in ways that aid the survival of pathogen.

## $T_h1/T_h2$ Cytokines Balance Determines the Disease Outcome

The progression of the diseases depend upon the balance between $T_h1$ cytokines (IL-2, IFN-γ and TNF-α) and $T_h2$ cytokines (IL-4, IL-5 and IL-10). Leprosy is not a single clinical entity; rather the disease presents, as a spectrum of clinical illness and the two major forms of the disease are tuberculoid and lepromatous. In tuberculoid leprosy, the CMI is in a heightened state and is characterized by granuloma formation. The response is $T_h1$ type being dependent on $T_h1$ cytokines. Where as the lepromatous leprosy, the CMI is in diminished state being guided by $T_h2$ types of response with high level of IL-4, IL-5 and IL-10. There is increase in the level of antibodies.

It is well-documented fact that there are changes in the $T_h$ subset activity in HIV infection. Early in the disease process, $T_h1$ activity is high. As the disease progresses to full-blown acquired immune deficiency syndrome (AIDS), the $T_h1$ cell activity is replaced by $T_h2$ activity.

## Cytokine Related Diseases

Several diseases can result from overexpression or underexpression of cytokines or cytokine receptors are as follows.

**Table 9.3** Major properties of human non-interleukin cytokines

| Cytokine | Principal cell source | Principal effects |
|---|---|---|
| TNF-α[*] | Activated macrophages, other somatic cells | IL-1[†] like effects, vascular thrombosis and tumor necrosis. |
| TNF-β | Activated $T_h$1 cells | IL-1 like and TNF-α like effects. |
| IFN-α[‡] and β | Macrophage, neutrophils, etc. | Antiviral effect, induction of class I MHC[§] on somatic cells, activation of macrophages and NK[‖] cells |
| IFN-γ | Activated $T_h$1 and NK cells | Induction of class I MHC on all somatic cells; induction of class II MHC on APCs[¶]. Activation of macrophages neutrophils and NK cells; promotion of CMI[**] (inhibits $T_h$2 cells); antiviral effect (weak) |
| CSF[††]<br>(G-CSF, M-CSF, GM-CSF[§§], EPO[‖‖], TPO[¶¶], SCF[***]) | Monocytes, macrophages fibroblasts, T lymphocytes | These cytokines help the production of particular mature blood cell types from pluripotent stem cells |
| TGF[†††]-B | Activated T lymphocytes, platelets, macrophage, etc. | Anti-inflammation<br>Antiproliferation of stem cell<br>Wound healing by promoting fibroblast proliferation |
| LIF[‡‡‡] | T cells | Proliferation of stem cell<br>Eosinophil chemotaxis |

[*] TNF-α, tumor necrosis factor-alpha; [†] IL-1, interleukin-1; [‡] IFN, interferon; [§] MHC, major histocompatibility complex; [‖] NK, natural killer; [¶] APCs, antigen-presenting cells; [**] CMI, cell-mediated immunity; [††] CSF, colony-stimulating factor; [§§] GM-CSF, granulocyte-macrophage colony-stimulating factor; [‖‖] EPO, erythropoietin; [¶] TPO, thrombopoietin; [***] SCF, stem-cell factor; [†††] TGF, transforming growth factor; [‡‡‡] LIF, leukemia inhibitory factor.

### *Bacterial Septic Shock*

Bacterial septic shock following prolonged infection by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes and Neisseria meningitidis,* results in overproduction of cytokines. The endotoxins stimulate macrophage to produce large quantity of IL-1 and TNF-α. The symptoms include a drop in blood pressure, fever, diarrhea and widespread blood clotting in various organs.

### *Bacterial Toxic Shock Syndrome Caused by Superantigens*

Bacterial superantigens consist of several enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 (TSST-1) from *Staphylococcus aureus*, pyrogenic exotoxins from *S. pyogenes*. Large number of T cells, activated by these superantigens result in excessive production of cytokines.

### *Lymphoid and Myeloid Cancers*

Abnormal cell proliferation of cancer cells may occur, as a result of overproduction of cytokines or their receptors. There is a evidence that abnormally high level of IL-6 is secreted by myxoma cells, myeloma cells, cervical and bladder cancer cells. In myeloma cells, IL-6 appears to operate in an autocrine fashion, stimulating excessive cell proliferation.

### *Chagas' Disease*

Chagas' disease is a protozoan disease caused by *Trypanosoma cruzi*. The disease is characterized by severe immune suppression due to lack of activation of $T_h$ cells. The defect, in this disease, may be attributes to substantial reduction in the expression of the $\alpha$-subunit of IL-2 receptor.

## Overview and Prospects

The cytokines in general, serve as a crucial intercellular messengers engaged in host defense, tissue repair and many other essential functions. The same cytokine can be produced by multiple cell types and can have multiple effects on the same cell and also can act on many different cell types. Their effects are mediated by specific receptors on target cells and these receptors may provide a useful therapeutic target for modulating cytokine activity. Owing to the complexity of the cytokine interactions, their use in the therapy is very limited.

A few cytokines, notably interferon and GM-CSF have proven to be therapeutically useful. The main constraints of adapting cytokine therapy are:

1. Difficulty in maintenance of effective dose level during therapy.
2. Very short half-life.
3. Unpredictable and undesirable side effects.

With further research on cytokines, the agonists and antagonists and their receptors, the management and treatment of inflammation, infection, allergy, autoimmune diseases and neoplastic diseases will be possible.

# THEORIES OF IMMUNE RESPONSE

One of the strange phenomena about the antibody molecule, the immunologists faced, is its specificity to foreign material or antigen. The antibody, so produced reacts specifically with the antigen, which produced it. The work of Karl Landsteiner and others showed that injecting an animal with almost any organic chemical could induce production of antibodies that would bind specifically to it. Various studies indicated that antibodies have a capacity for an unlimited range of reactivity. Two major theories were put forward to explain the various features of immune response, such as specificity and memory. They are instructive theories and selective theories. Instructive theories were proposed by chemists, who were more concerned with explaining the physicochemical aspects of specificity, than with the biological principles of immune process. The selective theories give more emphasis on immunocomplement cells rather than the antigen. The selective theories postulate that immunocomplement cells have only a restricted immunological range. The antigen exerts a selective influencing effect by picking up a appropriate immune complement cell to proliferate and synthesize specific antibody.

## Side-chain Theory

The earliest conception of selective theory was proposed by Ehrlich in 1900. According to Ehrlich, each cell would make a large variety of surface receptors, which bound foreign antigens by complementary shape lock and key fit. Exposure to antigen would provoke overproduction of receptors (antibodies), which would then be shed into the circulation. This theory explains well the specificity of the antibody response. This theory was abandoned subsequently, following Landsteiner's discovery that antibodies could be formed not only against natural antigen, but also against various synthetic chemicals.

## Template Theories

In the 1930s and 1940s, the selective theory was challenged by various instructive theories in which antigen plays a central role, in determining the specificity of antibody molecule. According to these instructive theories, a particular antigen would serve as a template around which the antibody would fold. The antibody molecule would there by assume a configuration complementary to that of antigen template. This concept was first postulated by Friedrich Breinl and Felix Haurowitz (1930). This theory was known as direct template theory.

Burnet and Fenner (1949), postulated that the entry of the determinant into the APC induced in it a heritable change. A genocopy of the antigenic determinant was incorporated in genome and transmitted to the progeny cell. This theory was designated as indirect template theory, which explained specificity and secondary response, but became unacceptable with the advances in molecular biology and protein synthesis.

## Selection Theories

In 1930 thereafter, the selective theories resurfaced. Jerne, reintroduced the concept of selective function of antigen in his natural selection theory. According to Jerne, million of globulin (antibody) molecules were formed in embryonic life, which covered the full range of antigenic specificities. These antibodies were natural antibodies. When antigen was introduced, it combined selectively with globulin, which had the nearest fit and entered into the antibody-forming cell (AFC) and directed the AFC to synthesize same kind of antibody. In this theory, selection was postulated in the level of antibody molecule, but not in the cell.

Burnet in 1957, argued that immunological specificity existed in the cell, not in the serum and proposed the most acceptable clonal selection theory. According to this theory, an individual lymphocyte expresses membrane receptor that is specific for a distinct antigen. This unique receptor specificity is determined before the lymphocyte is exposed to antigen. Binding of antigen to its specific receptor activates the cell, causing it to proliferate into a clone of cells that have the same immunological specificity as the parent cell. The clonal selection theory has been further redefined and presently accepted as the theory, which satisfies many of the features of immune responses. Clonal selection provides a frame work for understanding the specificity, immunological memory and self/non-self recognition characteristics of adaptive immunity.

## Jernes Network Hypothesis

Jerne postulated this hypothesis to explain the mechanism of regulation of antigenic combining site that is different in different antibodies. The unique amino acid sequences of variable region of heavy chain ($V_H$) and variable region of light chain ($V_L$) domains of a given antibody can function not only as an antigen-binding site, but also as a set of antigenic determinants. Each individual antigenic determinant of the variable region is referred to as an idiotope. In some cases, an idiotope may be the actual antigen-binding site and in some cases, an idiotope may comprise variable region sequences outside the Abs. Each antibody will contain multiple idiotopes, the sum of the individual idiotopes is called idiotype of the antibody. The idiotype may serve as an antigenic determinant and their may be a formation of anti-idiotypic antibodies. This in turn can induce antibodies to them and so on, forming a idiotype network, which is postulated to regulate the antibody synthesis and the number of antibody-forming cells in action.

Genetic basis of antigenic diversity has been clarified to a greater extent. Millions of antibody molecules exist in our body. If the theory of one gene-one antibody molecule holds good, then there would have been

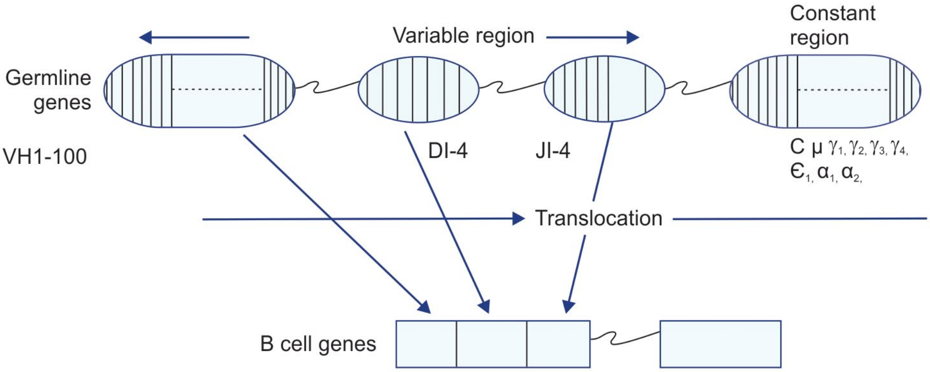


**Fig. 9.23:** Illustration of gene shuffling to form a B cell encoding for IgG1 heavy chain with $V_5$, $D_3$, J variable region sequence (similar shuffling also occurs for light chains—not shown).

millions of genes concerned in antibody production. This is obviously impossible. The phenomenon of split genes explains this. The genetic information for the synthesis of immunoglobulin molecule is not present in a continuous array of codons. Instead, this information occurs in several discontinue stretches of DNA, each coding for separate regions of antibody molecule. As the constant regions are identical for immunoglobulins of any one type, there need be only one gene or a few genes for each constant region, as against a very large number of genes for the variable genes. For example, the kappa L chain genes are composed of three separate segments V, J and C. There are about a hundred different types of variable (V) domine sequences and only one constant (C) segment, with some five joining (J) segment in between. By combining different V and J sequences with the C domain, it is possible to provide for antibodies with at least 500 different specificities. By palindromic arrangement (sequences that can be attached by either end), it is possible to generate many times more different specificities. The lambda chain has additional C sequences. The heavy (H) chain gene has also a diversity (D) segment. By the shuffling of these different segments of the light (L) and H chains, it is possible to have antibodies with far more than $10^8$ types of specificities. The split gene shuffling takes place during cell development and a mature B cell DNA will have only one combination of the different segments of the immunoglobulin gene and therefore, can produce only one type of antibody (Fig. 9.23).

The discovery of split genes for immunoglobulin demolished the long-standing understanding of one gene-one protein theory in genetics and has important implications in biology, beyond immunology. For this discovery, Susumu Tonegawa was awarded the Nobel prize in medicine in 1987.

## STUDY QUESTIONS

### Essay Questions

1. What happens when B cell responds to a foreign antigen? How do functions of plasma cells and memory cells differ?
2. How do the primary and secondary responses to the same antigen differ? What is the significance of those differences?

3. Mention various theories of immune response. Describe the clonal selection theories.
4. Define monoclonal antibodies. How monoclonal antibodies can be produced?
5. How do humoral immune response eliminate bacteria, viruses and toxins?
6. Name the cells, which are involved in cell-mediated immunity. What role do macrophages and dendritic cells play in CMI?
7. Explain the function for the following types of cells: $T_c$, $T_h1$, NK cells, eosinophils. What is a cytokine?

## Short Notes

1. Secondary response.
2. B cells.
3. T cells.
4. Cytokines.
5. Interleukins.
6. Tumor necrosis factor.
7. Interferons.
8. CD4 cells.
9. CD8 cells.
10. T-dependent antigens.
11. T-independent antigens.

## SUGGESTED READING

1. Biron CA. Role of early cytokines, including alpha and beta interferons (IFN-$\gamma$), in innate and adaptive immune responses to viral infections. Semin Immunol. 1998;10:383.
2. Black JG. Microbiology Principles and Applications, 3rd edition. USA: Prentice Hall college div; 1996.
3. Colligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology, New York: Wiley; 1997.
4. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
5. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
6. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
7. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
8. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby: Elsevier; 2006.
9. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
10. Tortora, Funke, Case. Microbiology an Introduction, 6th edition; 1998.

# Immunological Tolerance

# 10

Immunological tolerance is a state of unresponsiveness to a particular antigen to which a person has been exposed earlier. The important aspect of tolerance is the self-tolerance, which prevents the body to mount immune response against self-antigens. Since, the immune cells (lymphocytes) possess vast diversities of antigen receptors, it is possible that some receptors may be self-reactive.

The first evidence of self-tolerance was introduced by Traub in 1938, who inoculated mice, *in utero*, with lymphocytic choriomeningitis virus producing infection and maintained it throughout life. These inoculated mice, unlike normal mice did not produce neutralizing antibodies against the virus. Subsequently, Owen in 1945, described an experiment of nature in dizygotic (non-identical twins) cattle. Each of the twins had erythrocytes of its own and others blood group, as there is exchange of blood via their shared placental blood vessel. They exhibited lifelong tolerance to the other foreign cells, i.e. the relevant erythrocyte antigens. Burnet and Fenner in 1949, postulated that time of encounter of the antigen was critical in determining the responsiveness. When the immune cells are immature, during development in intrauterine life, whatever antigenic epitopes they encounter, they think as their own and do not respond afterwards during life.

They also postulated that tolerance could be induced, if some foreign antigens are administered during embryonic life and also in neonates. Medawar and his colleagues in 1953, further supported the experiment inducing immunological tolerance to skin allografts (grafts that are genetically not similar) in mice by neonatal injection of allogeneic cells. Hence, the key factor determining the tolerance is not the developmental stage, but the state of maturity of the immune cells (lymphocytes) at the time of the encounter of the antigen. In unborn and neonates, the immune cells are still to mature and therefore, the individual remains unresponsive at this stage.

## INTRATHYMIC TOLERANCE TO SELF-ANTIGENS

In the thymus, proliferation and massive death of thymocytes takes place simultaneously. A large chunk of double positive cell [cluster of differentiation ($CD4^+$), $CD8^+$] die within the thymus. Among the factors, which account for, are aberrant T cell receptor *(TCR)* gene arrangements, negative selection and failure to be positively selected.

In positive selection, T cells that have some degree of binding avidity (low levels of α, β and TCR) in the polymorphic groove of the major histocompatibility complex (MHC) molecule are selected for survival.

This binding is presumed to protect the cell from programmed cell death. This positive selection ensures that mature T cells recognize peptides, only in the cleft of MHC molecule and so, will be MHC restricted. Therefore, there must be some negative selections, which operate simultaneously to silence the self-reactive lymphocytes.

Since, both the positive selection and negative selection requires the association of TCR with MHC-peptide complex, what led to choose positive or negative selection? The difference between these two processes is related to the avidity of the engagement, which is a function of the affinity of TCR with its MHC-peptide complexes and the concentration of epitope. A stronger engagement leads to negative selection and clonal deletion is the predominant mechanism of negative selection. Negative selection depends on various factors such as accessibility of developing T cells to self-antigen, the combined avidity of TCR and accessory molecules ($CD8^+$ or $CD4^+$) for the self-MHC, self-peptide complex and the identity of the deleted cells. Negative selection does not require specialized antigen-presenting cells (APCs). It is normally a function of macrophages or thymic dendritic cells, which are rich in MHC I and MHC II and are situated predominantly in the corticomedullary junction. These cells can bind the cells that have high avidity for self-peptides. Thymic cells are also involved in negative selection.

## POST-THYMIC TOLERANCE TO SELF-ANTIGENS

Despite the thymic surveillance, some of the autoaggressive self-reactive lymphocytes escape negative selection and circulate in the blood of healthy individuals. These circulate because the T cells TCRs have too lower affinity for self-peptide MHC complex displayed on thymic stromal cells or because the concentration of surface MHC peptide complex is too low.

If such autoaggressive cells are in the circulation, what prevents autoimmunity? Self-reactive cells, some times ignore the presence of self-antigen. The circumstances in which the presence of antigen is ignored firstly, by the inaccessibility of the reactive cells to the site of antigen (sequestered antigen). Secondly, even if they have access to the site, cannot be activated, because of:

1. Low concentration of antigen.
2. The presence of self-antigen in tissue cells that express few or no MHC molecules.
3. When they do not get help in the form of cytokines from other cells.

Self-reactive cells also can be tolerized by self-antigen on tissue cells or silenced by immunoregulatory cells. Anergy is a term refers to the failure of T cell response *in vivo* and *in vitro* due to lack of costimulatory signal. When T cells are presented with antigens on non-professional APCs, there is no delivery of costimulatory signals, due to lack of molecules such as B7-1 and B7-2. These costimulation molecules bind on the T cells' CD28 and CTLA 4 molecule.

Certain lymphokines such as transfer growth factor-beta (TGF-β) produced by T cells suppress immune response of self-reactive cells. Further, the two helper T cells ($T_h1$) and $T_h2$ produce cytokines, which antagonize each other and play a role in immune regulation (Fig. 10.1). For example, a $T_h2$ response to a particular self-epitope may produce little or no pathology, but a $T_h1$ response may cause an injurious cell-mediated inflammatory response such as delayed hypersensitivity reaction. As a result, the overt autoimmune disease may be determined by the relative balance between $T_h1$ cytokines [interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor (TNF)]

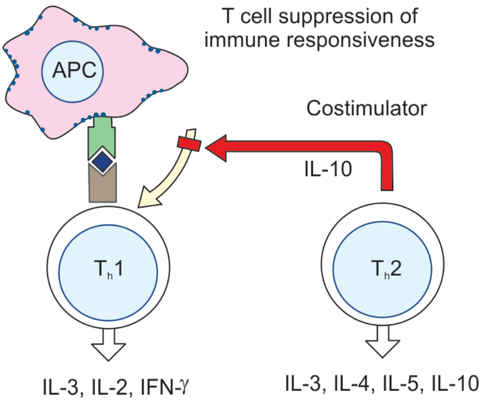


**Fig. 10.1:** Two subset of cells, $T_h1$ and $T_h2$, exist. Each subset has a distinct pattern of cytokine production (white arrows). Through their production of IL-10, the $T_h2$ cells may render $T_h1$ cells anergic by interfering with the costimulator function of antigen-presenting cells (APCs).

and $T_h2$ cytokines [IL-4, IL-10 transforming growth factor-beta (TGF-β)]. Changes that favor the development of $T_h1$ like cell-mediated inflammatory response, perhaps triggered by pathogenic bacteria, may be the basis for autoimmune inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis.

Tolerance to self-epitopes can also be induced by regulatory cells. In most cases, the regulatory cells are T cells. $CD4^+$ and $CD25^+$ T cells diminish the activity of T cells stimulated by a variety of epitopes. They play important role in preventing inflammatory diseases (e.g. IBD). Some $CD8^+$ T cells also inhibit the activation and proliferation of $CD4^+$ preventing autoreactive type-IV hypersensitivity, delayed-type hypersensitivity (DTH) reaction. $CD8^+$ and $CD4^+$ 'T'-cell subpopulation has been seen inhibiting antibody production.

## B CELL TOLERANCE TO SELF-ANTIGENS

The B cells that make autoreactive immunoglobulins are silenced in two main ways.

The first applies to the situation in which an immature B cell contacts with antigen in the bone marrow and secondly, when a mature B cell comes in contact with antigen outside the bone marrow.

It has long been established that when an immature B cell comes in contact with the self-antigen, there is cross-linking of membrane immunoglobulin M (mIgM) leading to the death of the cells in the bone marrow (negative selection).

Later work by other researchers using transgenic system described by Nemazee and Biirki, showed the negative selection of immature B cells, does not always result in their immediate deletion. Instead, maturation of the self-reactive cell is arrested, while the B cell will 'edit' its receptor.

The cells upregulate the recombination activating gene-1 *(RAG-1)* and *RAG-2* recombinase protein expression, so that they are able to resume rearranging their light chain genes. Some of the cells succeed in replacing the kappa (K) light chain of the self-antigen reactive antibody with a lambda (λ) chain encoded by endogenous chain gene segments. As a result, these cells will begin to express an 'edited' mIgM with a different light chain and specificity that is not self-reactive. Because these cells are no longer self-reactive, they will escape negative selection and leave the bone marrow as mature B cells bearing the edited non-self-reactive mIgM.

The receptor editing mechanism is applicable only to immature B cells, which confront the antigens (serum proteins, cell surface or extracellular matrix proteins) in the bone marrow. Different mechanisms holds good to deal with the self-antigens, which are present outside the bone marrow. They are as follows:

1. When a mature B cell contacts antigen in the absence of appropriate T cell co-operation, there is death of the B cell

or B cell fails to carry out immune response. But autoantibodies can be produced, if an anti-self B cell collaborates with an anti-non-self $T_h$ cell in response to cross-reactive antigens containing both self and non-self determinants. For example, some microorganisms have some cross-reactive antigens that have both foreign T cell reactive epitopes and other epitopes that resemble self-epitopes and are capable of stimulating B cells. Such antigens could provoke a vigorous antibody response to self-antigens (Fig. 10.2).

2. In contrast to TCRs, the immunoglobulin receptors on mature antigenically stimulated B cell can undergo hypermutation and may acquire anti-self reactivities at the late stage. Tolerance may thus, be imposed on B cell both during their development and after antigenic stimulation in secondary lymphoid tissue.
3. The fate of the B cell depends on the physical nature of the antigen involved. In the case of membrane bound or particulate antigens, the self-reactive cell dies and this is known as clonal deletion. Soluble protein antigens, which give weaker signal to B cell receptor (BCR) of self-reactive B cells presumably make the cell unresponsive, which is known as clonal anergy. The anergic cell can be activated under some circumstances. The longevity of the anergic cell is less therefore, they die early.

## ARTIFICIALLY INDUCED TOLERANCE *IN VIVO*

Tolerance can be produced in various ways as follows:

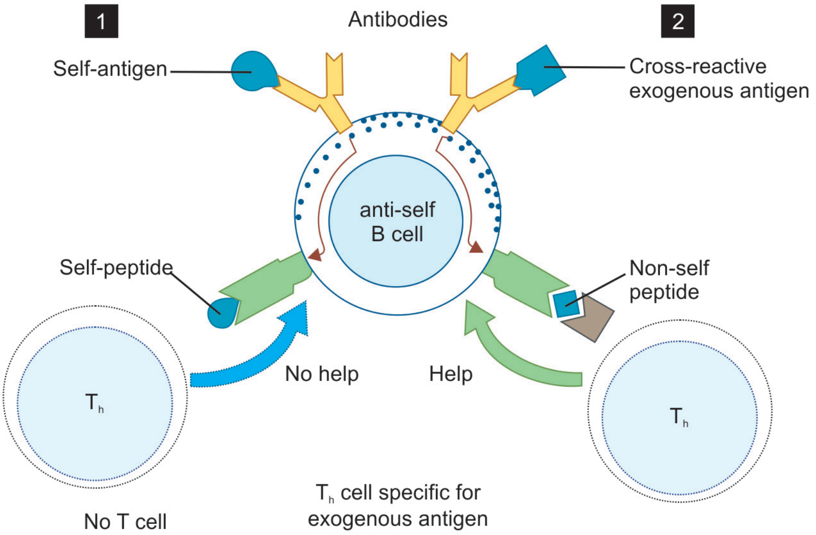


**Fig. 10.2:** Loss of B cell tolerance to cross reactive antigen. **1.** Tolerance of self-epitopes. If $T_h$ cells are not available, either because of a hole in the T cell repertoire or because of deletion resulting from self-tolerance achieved intrathymically, any B cells that are self-reactive (anti-self) will nevertheless be unable to mount an anti-self antibody response; **2.** Autoantibodies can be produced, if an anti-self B cell collaborates with an anti-non-self $T_h$ cell in response to cross-reactive antigens containing both self and non-self determinants.

1. Tolerance occurs in chimerism: Tolerance can be induced by inoculation of allegonic cells into the hosts, which lack immunocompetence. For example, in neonates, in adults, which are undergoing immunosuppression regimens (whole body irradiation, cytotoxic drugs such as cyclosporine, antilymphocyte antibody, etc.). For tolerance to be maintained certain degree of chimerism (coexistence of cells from two genetically different individuals) must be produced.
2. Antibodies to T cell coreceptors induce tolerance. Monoclonal antibodies against CD4 and CD8 molecules cause tolerance of respective cells.
3. Soluble antigens induce tolerance. Tolerance is inducible in neonates and adults by administering soluble protein antigens in deaggregated form. Susceptibility to tolerization differs in T cells and B cells. Thus, tolerization is achieved in T cells from spleen and thymus, after very low dose within few hours. The tolerance of spleen B cells requires much more time and more antigen dose.
4. Oral administration of antigens induces tolerance by some mechanism that may involve immunoregulatory and suppressor cell network generated in the intestinal wall.
5. Tolerance can be achieved by targeting the antigen to the naive B cell. B cell processes antigen and presents the MHC-peptide complex to T cell. The B cell lacks B7, hence lacks costimulatory molecule.
6. Extensive clonal proliferation by repeated antigenic challenge can lead to exhaustion and tolerance.
7. Antagonist peptides that signal inappropriately induce tolerance. When the antagonist peptides fit into the antigen binding groove of MHC transmits negative signal or partial signaling (Refer Fig. 10.3).

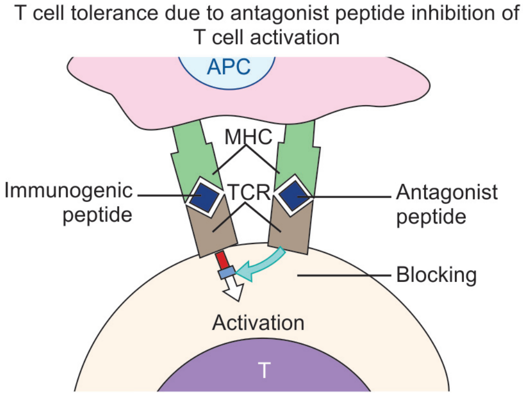

T cell tolerance due to antagonist peptide inhibition of T cell activation
APC
MHC
TCR
Immunogenic peptide
Antagonist peptide
Blocking
Activation
T

**Fig. 10.3:** Antagonist peptides and immunogenic peptides both bind to the major histocompatibility complex (MHC) molecules on professional antigen-presenting cells (APCs). The interaction of the TCR with the MHC-antagonist peptide prevents stimulation by the immunogenic ligand through an inhibitory effect on the signaling pathways.

8. Slowly metabolized T cell-independent antigens induce B cell tolerance. In very high concentration of T-independent antigens, it blockade the surface receptor of the fully differentiated antibody-forming cells (AFCs) and present them from secreting antibody.
9. Anti-idiotype antibody can induce B cell tolerance. An antibody's combining site (hypervariable region) may act as an antigen and induce the formation of antibodies. These antibodies, by cross-linking immunoglobulin on B cell can cause B cell unresponsiveness.
10. Manipulation of selective immune response by cytokines.

$T_h1$ type of helper T cells are most active in cell-mediated immune responses, such as delayed hypersensitivity and foreign graft destruction. Interference with their function by inducing $T_h2$ cells may be beneficial in transplanted systems. For example, IL-10, a cytokine produced by $T_h2$ cells can suppress the activity of $T_h1$ cells by an effect on APCs and there by diminish the damage caused by $T_h1$ cells.

## STUDY QUESTIONS

### Essay Questions

1. What is immunological tolerance? Describe about the thymic tolerance to self-antigens.

### Short Notes

1. B cell tolerance.
2. Clonal deletion.
3. Clonal anergy.

## SUGGESTED READING

1. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
2. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
3. Jawetz Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
4. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby Elsevier; 2006.
5. Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical book); 2007.
6. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's Illustrated Reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
7. Tortora, Funke, Case. Microbiology; An Introduction, 6th edition. USA; 1998.

# Hypersensitivity

## 11

In response to antigenic stimulation, the immune system of the body responds differently. An immune response evokes a battery of effector molecules that act to remove the antigen by various mechanisms, which has been discussed in previous chapters. Generally, these effector molecules induce a subclinical localized inflammatory response that eliminates antigens without producing extensive tissue damage. Under certain circumstances, however, the inflammatory reactions produce deleterious effect on the host leading to significant tissue damage or sometimes, even death. This inappropriate immune response is termed as hypersensitivity or allergy.

## DEFINITION

The term hypersensitivity refers to those inappropriate immunological reactions, which rather than contributing to recovery, themselves produce tissue damage and forms an important and sometimes, a major part of the disease process.

Hypersensitivity reactions can be provoked by many antigens; the cause of hypersensitivity reaction will vary from one individual to the next. Hypersensitivity is not manifested in first contact with the antigen, but usually appears on subsequent contact.

## GELL AND COOMBS CLASSIFICATION

Gell and Coombs proposed a classification scheme in which hypersensitivity reactions are divided into four types, I, II, III and IV each involving distinct mechanisms, cells and mediator molecules.

Exposure to antigen ⟶ Immunity

OR

Exposure to antigen ⟶ Inappropriate immune reactions (hypersensitivity)

### Classification

The hypersensitivity is classified into two types, i.e. immediate hypersensitivity and delayed hypersensitivity.

1. Immediate hypersensitivity: This type of hypersensitivity includes the following:
    - Type I: Anaphylactic or atopic reaction
    - Type 2: Cytolytic or cytotoxic reaction or antibody cell surface reaction
    - Type 3: Immune complex reaction.
2. Delayed hypersensitivity: This type of hypersensitivity include the following:
    - Type 4: Hypersensitivity.

Type I, II and III reactions depend on interaction of antigen with humoral antibody and tend to be called immediate type hypersensitive reactions although, some are more immediate than others. Type 4 reaction involves T cell recognition and because of longer time course, this is referred to delayed type of hypersensitivity reaction.

## IMMEDIATE TYPE I HYPERSENSITIVITY

Type I hypersensitivity reaction is induced by certain types of antigens known as allergens and has all the hall marks of a normal humoral response. The allergens induce a humoral response by the same mechanism, as antigens generating antibody-secreting plasma cells and memory cells. But the only difference is that in response to allergens, the plasma cells secrete immunoglobulin E (IgE). This class of antibody is cytophilic in nature and has got high affinity for the fragment crystallizable (Fc) receptors on the surface of the tissue mast cells and basophils. Such an IgE coated mast cell or basophil is known as sensitized cell. A subsequent exposure to same allergen cross-links the membrane bound IgE on the sensitized mast cell or basophil causing degranulation of these cells (Fig. 11.1).

Following degranulation, there is liberation of pharmacologically active mediators to the surrounding tissues. The main actions of these mediators are vasodilation and smooth muscle contraction.

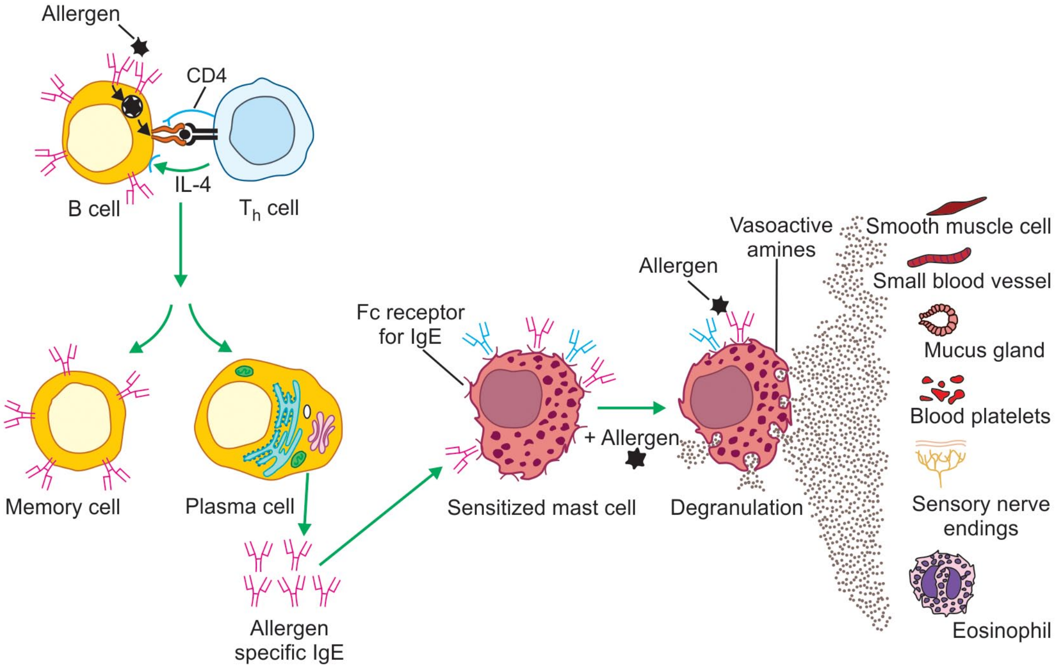

Allergen
CD4
IL-4
B cell
$T_h$ cell
Memory cell
Plasma cell
Allergen specific IgE
Fc receptor for IgE
Sensitized mast cell
+ Allergen
Allergen
Degranulation
Vasoactive amines
Smooth muscle cell
Small blood vessel
Mucus gland
Blood platelets
Sensory nerve endings
Eosinophil

**Fig. 11.1:** General mechanism underlying a type 1 hypersensitive reaction. Exposure to an allergen activates B cells to form IgE secreting plasma cells. The secreted IgE molecules bind to IgE specific Fc receptors on mast cells and blood basophils (many molecules of IgE with various specificities can bind to the IgE specific Fc receptor). Second exposure to the allergen leads to cross-linking of the bound IgE, triggering the release of pharmacologically active mediators, such as vasoactive amines from mast cells and basophils. The mediators cause smooth muscle contraction, increased vascular permeability and vasodilatation.

## Components of Type I Reactions

Several components are necessary for the development of type I hypersensitivity reactions.

### *Allergens*

Immunoglobulin E is produced in large quantity as a defence against parasitic infection. The level of IgE remains high, as long as the parasite survives in the host. Some people may have an abnormality called atopy, a hereditary predisposition to the development of immediate type of hypersensitivity reaction against common environmental antigens. In this condition, abnormally there is high IgE production against non-parasitic antigens. Hence, the term allergen refers specifically to the non-parasitic antigens capable of stimulating type I hypersensitivity reaction in all sensitized individuals.

The high level of IgE is partly genetic, but often familial. Along with high level of IgE, there is also marked degree of eosinophilia. The genetic propensity of atopy is governed by at least genes in two loci, one is situated on chromosome 5 and is responsible for the production of cytokines including IL-3, IL-4, IL-5, IL-9, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF). A second locus on chromosome 11 is linked to a region that encodes the β chain of high-affinity IgE receptors.

Most IgE responses occur on the mucosal surface and are either inhaled or ingested. The common allergens are proteins (foreign serum, vaccine), plant pollens (ryegrass, ragweed, timothy grass, etc.), drugs (penicillin, sulfonamides, salicylates), food (nuts, seafood, eggs, pea, beans, milk), insect products (bee venom, wasp venom, dust, mites and cockroach calyx), mold spores and animal hair and dander.

It appears that allergenicity is a consequence of a complex series of interactions involving not only the allergen, but also the dose, the sensitizing route, sometimes an adjuvant, but most important is the genetic constitution of the recipient.

### *Reaginic Antibody (IgE)*

Allergy is mediated by IgE. Prausnitz and Kushner (1921) were first to describe the mechanism of allergic reaction. The serum from Kushner (who was allergic to fish) was injected to the skin of Prausnitz. When subsequently the fish antigen was injected to the sensitized site, there was an immediate wheel and flare reaction. Prausnitz and Kushner proposed the existence of atopic reagin in the allergic individuals. There after Ishizaka showed that the atopic reagin is a new class of immunoglobulin called IgE.

### *Mast Cells and Basophils*

Mast cells are found throughout connective tissue particularly, near blood and lymphatic vessels. High concentrations of mast cells are also found in many other tissues such as skin, mucous membrane of gastrointestinal tract and respiratory tract. The electron micrograph of mast cells reveal numerous membrane-bound granules, which contain pharmacologically active mediators. Basophils circulate in the bloodstream, where they constitute less than 1 percent of the leukocytes. Electron micrograph reveals a multilobed nucleus, few mitochondria, numerous glycogen granules and electron-dense membrane-bound granules, which contain pharmacologically active mediators. After activation, these mediators, in both mast cells and basophils are released causing the clinical manifestations of the type 1 hypersensitivity reactions.

### *Immunoglobulin E-binding Fc Receptors*

The binding activity of IgE depends on the presence of receptors specific for the Fc region of the ε (epsilon) heavy chain. These receptors are of two types, they are designated as FcεRI and FcεRII. Any mast cell or basophil may contain either of these two receptors. FcεRI receptors are 1,000 time stronger in their affinity for IgE.

*High-affinity (FcεRI) receptor:* Enables it to bind IgE despite low serum concentration of IgE. Allergen-mediated cross-linkage of the bound IgE, results in aggregation of FcεRI receptors and rapid tyrosine phosphorylation, which initiates the process of mast cell degranulation.

*Low-affinity receptor of IgE (FcεRII):* It is also known as CD23, which is specific for $CH_3$/$CH_3$ domain of IgE and has a low affinity for IgE than does FcεRI. FcεRII plays a variety of roles in regulating the intensity of the IgE response. Allergen cross-linkage of IgE bound to FcεRII has been shown to activate B cells, alveolar macrophages and eosinophils.

### Mechanism of IgE-mediated Degranulation

Although, mast cell degranulation generally initiated by allergen cross-linkage of the bound IgE, a number of other stimuli can also initiate the process, including the anaphylatoxin (C3a, C4a, C5a); various drugs such as synthetic adrenocorticotropic hormone (ACTH), codeine and morphine and compounds such as an ionophore, compound 48/80, melittin (Fig. 11.2).

### Cross-linking of Receptor

Immunoglobulin E-mediated degranulation occurs, when an allergen cross-links IgE that are bound to FcεRI receptors on the mast cell or basophil. When the allergen attaches to one molecule of IgE (monovalent IgE) fixed on the mast cells, apparently there is no effect on the target cell. It is only after allergens cross-links the fixed IgE complex then degranulation occurs (Figs 11.3A to F).

Other experiments have shown that it is actually, the cross-linking of two or more FcεRI molecules with or without bound IgE is essential for degranulation of mast cells or basophils. Degranulation releases preformed mediators induces, synthesis of others from arachidonic acid.

The response is initiated when a multivalent antigen binds and cross-links two or more IgE antibodies occupying FcεRI receptors. This cross-linking transmits a signal that activates the mast cell, resulting in activation of protein tyrosine kinase and increases in intracellular free calcium levels. These signaling events complete soon after the antigen binding. Thereafter, cytoplasmic granules will fuse one another and also with the plasma membrane discharging their contents to the exterior.

Simultaneously, there is the induction of synthesis of newly formed mediators from arachidonic acid, leading to the production of prostaglandins and leukotrienes, which have a direct effect on local tissues. In the lung, they cause immediate bronchoconstriction, mucosal edema and hypersecretion leading to asthma.

### Mediators of Type I Reactions

The clinical manifestations, which occur in type 1 hypersensitivity reactions are due to the biological effect of the mediators released from the mast cell or basophil degranulation. These mediators have got action not only on the local tissue, but also on effector cells, which include neutrophils, eosinophils, T lymphocytes, monocytes and platelets. When these mediators initiate beneficial effect against parasitic infections, permitting the influx of plasma and inflammatory cells to the pathogen by vasodilatation and increased vascular permeability. On the other hand, the mediators released in response to allergen, unnecessarily increase the vascular permeability and inflammation causing tissue injury.

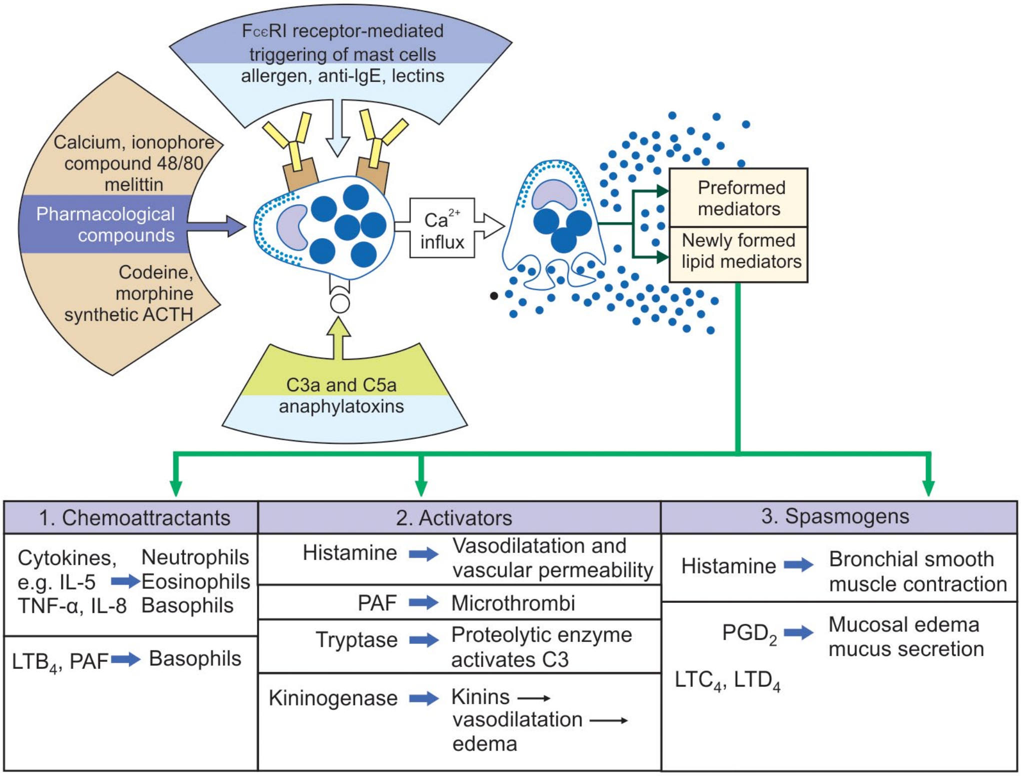
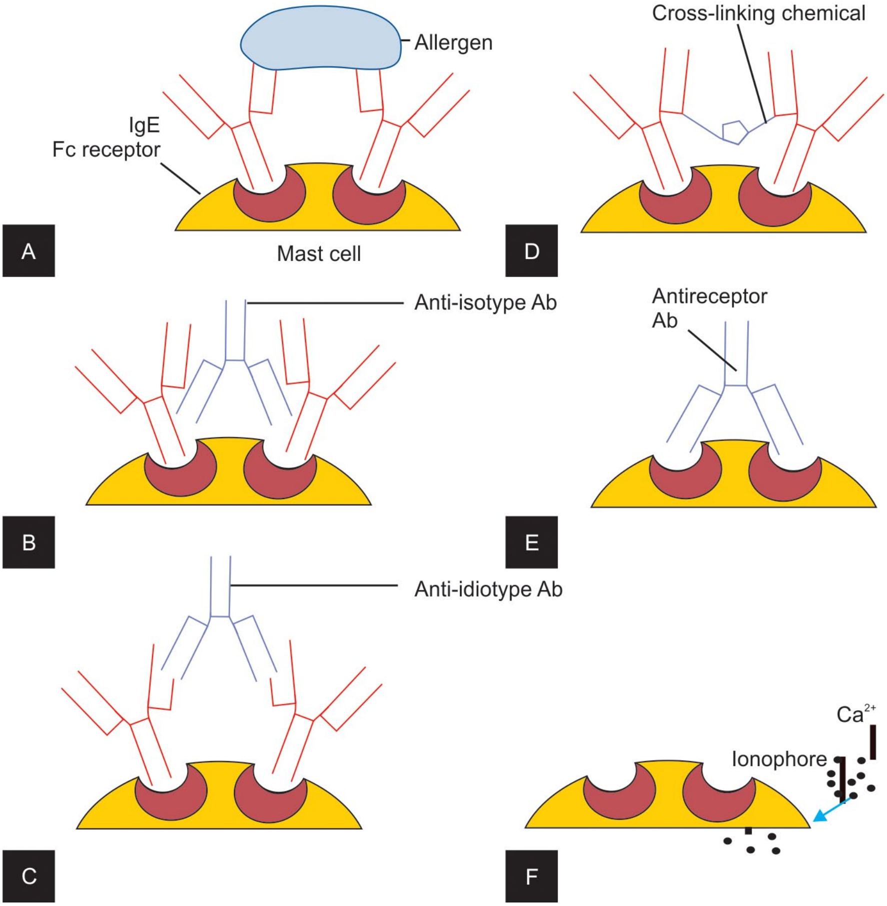

FcεRI receptor-mediated triggering of mast cells
allergen, anti-IgE, lectins
Calcium, ionophore compound 48/80 melittin
Pharmacological compounds
Codeine, morphine synthetic ACTH
$Ca^{2+}$ influx
C3a and C5a anaphylatoxins
Preformed mediators
Newly formed lipid mediators
1. Chemoattractants
Cytokines, e.g. IL-5 TNF-α, IL-8 → Neutrophils Eosinophils Basophils
$LTB_4$, PAF → Basophils
2. Activators
Histamine → Vasodilatation and vascular permeability
PAF → Microthrombi
Tryptase → Proteolytic enzyme activates C3
Kininogenase → Kinins → vasodilatation → edema
3. Spasmogens
Histamine → Bronchial smooth muscle contraction
$PGD_2$ → Mucosal edema mucus secretion
$LTC_4$, $LTD_4$

**Fig. 11.2:** Mast cell activation and physiological effects of mast cell-derived mediators. Mast cell activation can be produced by immunological stimuli, which cross-link Fc receptors and by other agents such as anaphylatoxins and secretagogues (e.g. compound 48/80, melittin, calcium ionophore, A23187). Some other drugs such as codeine, morphine and synthetic adrenocorticotropic hormone (ACTH) have also been found to act on mast cell directly. The common features in each case is the influx of $Ca^{2+}$ ions into the mast cell, which is crucial for degranulation. Microtubule formation and movement of the granules to the cell membrane lead to fusion of the granule with the plasma membrane and the plasma membrane associated with activation of phospholipase $A_2$, release of arachidonic acid; this can then be metabolized by lipoxygenase or cyclooxygenase enzymes, depending on the mast cell type. These newly formed lipid metabolites include prostaglandins ($PGD_2$) and thromboxanes produced by cyclooxygenase pathway and leukotrienes ($LTC_4$, $LTD_4$ and chemotactic LTB) produced by the lipoxygenase pathway. Both, the preformed granule-associated lipid mediators and the newly formed granule-associated lipid mediators have three main areas of action. **1.** Chemotactic agents: A variety of cells are attracted to the site of mast cell activation, in particular eosinophils, neutrophils and mononuclear cells including lymphocytes. In addition, recent evidence suggests that certain of the preformed cytokines released from degranulating mast cells are also chemotactic for inflammatory cells; **2.** Inflammatory activators: They can cause vasodilatation, edema and via platelet-activating factor (PAF), microthrombi, leading to tissue damage. Tryptase, the major neutral protease of human lung mast cells, can activate C3 directly; this function is inhibited by heparin. Kininogenase are also released and these affect small blood vessels by generating kinins from kininogens, again leading to inflammation; **3.** Spasmogens: They have a direct effect on bronchial smooth muscles, but could also increase mucus secretion leading to bronchial plugging.



**Figs 11.3A to F**: Schematic diagram of mechanisms that trigger degranulation of mast cells. **A.** Allergen cross-linkage of cell-bound IgE molecules; **B, C.** Antibody cross-linkage of IgE; **D.** Chemical cross-linkage of IgE; **E.** Cross-linkage of IgE Fc receptors by antireceptor antibody; **F.** Enhanced $Ca^{2+}$ influx stimulated by an ionophore that increases membrane permeability to $Ca^{2+}$ ions. Note that mechanisms the B, C and D do not require allergen; mechanisms of E and F require neither allergen nor IgE and the mechanism of F does not even require receptor cross-linkage.

The mediators may be classified as either primary or secondary (Table 11.1). Primary mediators are preformed, but stored in the granules (histamine, proteases, eosinophil chemotactic factor, neutrophil chemotactic factor and heparin). The secondary mediators are either synthesized following, cross-linking or released due to breakdown of phospholipids during granulation. They include platelet-activating factor, leukotrienes, prostaglandins, bradykinins and various cytokines. The main biological activities of some of the mediators are described subsequently.

*Histamine:* It is an inflammatory mediator and found preformed in the granules of mast cells and basophils. It is synthesized

**Table 11.1** Principal mediators involved in type 1 hypersensitivity

| Mediators | Effects |
|---|---|
| **Primary** | |
| Histamine | Increased vascular permeability, smooth muscle contraction |
| Serotonin | Increased vascular permeability, smooth muscle contraction |
| Eosinophil chemotactic factor of anaphylaxis (ECF-A) | Eosinophil chemotaxis |
| Neutrophil chemotactic factor of anaphylaxis (NCF-A) | Neutrophil chemotaxis |
| Proteases | Bronchial mucus secretion: Degradation of blood vessel basement membrane; generation of complement split products |
| **Secondary** | |
| Platelet-activating factor | Platelet aggregation and degranulation, contraction of pulmonary smooth muscles |
| Leukotrienes [slow-reacting substance of anaphylaxis (SRS-A)] | Increased vascular permeability contraction of pulmonary smooth muscles |
| Prostaglandins | Vasodilation contraction of pulmonary smooth muscle; platelet aggregation |
| Bradykinin | Increased vascular permeability, smooth muscle contraction. |
| Cytokines, interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) | Systemic anaphylaxis, increased expression of cell adhesion molecules (CAMs) on venular endothelial cells |
| IL-2, IL-3, IL-4, IL-5, IL-6, transforming growth factor-beta (TGF-β) and granulocyte-macrophage-colony-stimulating factor (GM-CSF) | Various effects |

from the amino acid histidine. Histamine once released after degranulation binds to receptors (H1, H2 and H3), present in different tissue of the body, most biologic effects are produced binding to H1 receptors. The binding induces contraction of intestinal and bronchial smooth muscles, increases permeability of the venules and increases mucus. Antihistamine drugs used to treat allergies, block the H1 receptor. In contrast, binding of histamine to H2 receptors augments gastric acid secretion and airways mucus production. Binding of histamine to H2 receptors on mast cells and basophils suppresses degranulation; thus, histamine exerts negative feedback on the release of the mediators. H3 binding of histamine affects synthesis and release of histamine.

*Arachidonic acid metabolites (Leukotrienes and prostaglandins)*: These are not formed until the mast cell undergoes degranulation and the enzymatic breakdown of phospholipids. Therefore, they are known as reaction mediators. An ensuing enzymatic cascade generates the prostaglandins and leukotrienes.

*Arachidonic acid:* It is a fatty acid, which can be liberated from membrane phospholipids by the action of phospholipase (phospholipase $A_2$). Once liberated, arachidonic acid can be metabolized by either the cyclooxygenase or lipoxygenase pathway. Each of

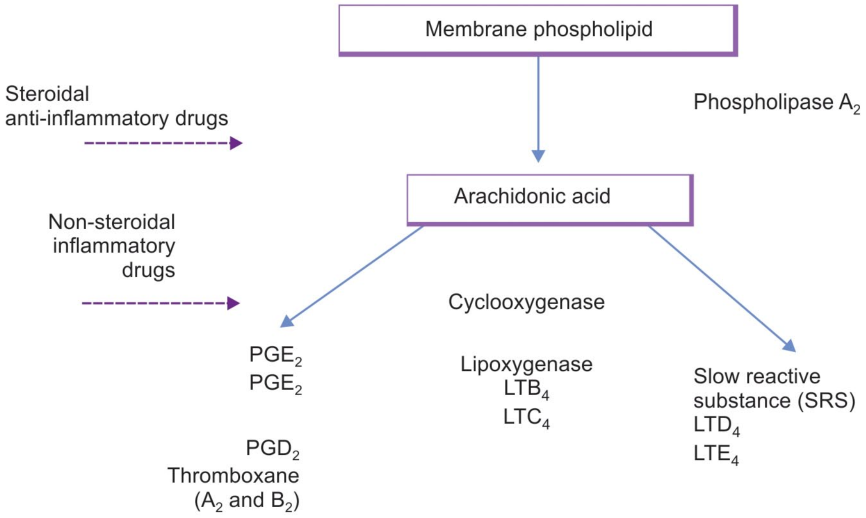

Membrane phospholipid
Phospholipase $A_2$
Steroidal anti-inflammatory drugs
Arachidonic acid
Non-steroidal inflammatory drugs
Cyclooxygenase
$PGE_2$
$PGE_2$
$PGD_2$
Thromboxane ($A_2$ and $B_2$)
Lipoxygenase
$LTB_4$
$LTC_4$
Slow reactive substance (SRS)
$LTD_4$
$LTE_4$

**Fig. 11.4:** Arachidonic acid pathway

these pathways can give rise to many alternative products (Fig. 11.4), each with its own spectrum of effects. The main products of cyclooxygenase and lipoxygenase pathways are respectively prostaglandins $D_2$ ($PGD_2$) and leukotrienes ($LTB_4$, $LTC_4$, $LTD_4$ and $LTE_4$). Their effects are more pronounced and long-lasting, however, than those of histamine.

The leukotrienes mediate bronchoconstriction, increased vascular permeability and mucus production. Prostaglandin $D_2$ causes bronchoconstriction. Being active at nanomole level leukotrienes are, as much as 1,000 times more potent as bronchoconstrictor than histamine.

*Cytokines:* Variety of cytokines released from mast cells and eosinophils, add to the complexity of type 1 hypersensitive reactions. Human mast cells secrete IL-4, IL-5, IL-6 and TNF-α. These cytokines, altering the local microenvironments, help to recruit neutrophils and eosinophils. IL-4 increases IgE by acting on B cell and IL-5 helps in recruitment and activation of eosinophils. The shock in systemic anaphylaxis is attributed to the secretion of TNF-α by mast cell.

## Factors Influencing Type 1 Reaction

The type 1 responses with increased level of IgE are dependent on several factors such as:

1. The effect of antigen dosage and mode of antigen presentation, influence the type of response and the production of the type of immunoglobulin. In an experimental study on mice, it has been shown that repeated low doses of an appropriate antigen induce persistent IgE response, but increasing dosage following repeated injections make a shift to IgG. The modes of antigen presentation with different adjuvants also influence the types of response. While immunization of Lewis strain rat with keyhole limpet hemocyanin (KLH) with aluminium hydroxide gel or *Bordetella pertussis,*

as an adjuvant, induce strong IgE response, where as KLH with complete Freund's adjuvant produces a largely IgG response.

2. A genetic component also plays an important role in influencing susceptibility to type 1 reaction in human being. A study shows that 50 percent of the offsprings suffer from allergy, when both the parents were allergic. In case, one of the parents is allergic, only 30 percent of the offsprings are liable to suffer from allergy. The genetic propensity of atopy is governed by at least genes in loci on chromosome 5 and 11.
3. The relative numbers of helper T cell 1 ($T_h1$) and $T_h2$ subsets are the most important factors in the regulation of type 1 response. $T_h1$ subsets, being guided by interferon-$\gamma$ (IFN-$\gamma$) reduces type 1 response and thereby, decreased IgE production and increase IgG production.

## Consequences of Type 1 Reactions

The term anaphylaxis was introduced by Charles Richet in 1902 to describe severe and fatal reactions of animals, sometimes to second protein.

The anaphylactic reaction may be systemic anaphylaxis and localized anaphylaxis (atopy).

### *Systemic Anaphylaxis*

Systemic anaphylaxis is a shock like and often fatal state, whose onset occurs within minutes of a type 1 hypersensitivity reaction. Systemic anaphylactic shock readily demonstrated in the guinea pig and animals particularly, sensitive to anaphylaxis. A guinea pig injected with an antigen protein such as egg albumin for the first time, experience no ill effects, but if reinjected intravenously, 10 to 14 days after, quite likely, the animal will die within few seconds from asphyxia caused by intense constriction of the bronchioles. Fortunately, anaphylactic shock in this scale is rare in human being, but does sometimes occur in response to drugs such as penicillin, insulin and antitoxins or following an insect sting (bee, wasp, hornet, etc.) or during desensitization with pollen extract. Epinephrine is the drug of choice for systemic anaphylactic reactions.

### *Localized Anaphylactic Reactions (Atopy)*

In localized anaphylaxis, the reaction is limited to specific target tissue or organs often involving epithelial surfaces, at the site of allergen entry. Localized anaphylactic reactions (atopy) are very common and include hypersensitivity to pollen, house dust, animal dander and food, which are familial, leading to allergic rhinitis (hay fever), allergic asthma, urticaria and food allergy respectively.

Localized anaphylaxis is caused by an antigen-antibody reaction in the tissue, at the surface of the mast cells followed by the release of mediators with pharmacological action on smooth muscle (contraction) and blood vessels (increased permeability).

### *Allergic Rhinitis*

Allergic rhinitis is the most common atopic disorder commonly known as hay fever. This occurs in response to airborne allergens with sensitized mast cells in conjunctiva and nasal mucosa, to induce the release of active mediators from the mast cells. The mediators cause localized vasodilation and increased capillary permeability leading to water exudation of conjunctival, nasal mucosa and upper respiratory tract, as well as sneezing and coughing.

### *Asthma*

Asthma is also a common manifestation of localized anaphylaxis. In some cases, pollens, dust, fumes, insect products, viral antigens, etc. serve as allergen triggering asthmatic attack (allergic asthma) type and in others, exercise or cold apparently independent of allergens may cause asthma (intrinsic asthma). In asthma, degranulation of mast cells and the mediators release occur in the lower respiratory tract, resulting contraction of bronchial smooth muscle. Airway edema, mucus production and inflammation contribute to bronchial constriction and airway obstruction.

Most clinicians consider asthma, primarily, an inflammatory disease. Asthmatic patients exhibit responses early and late. The early response occurs within minutes and primarily involves, histamine, leukotrienes and $PGD_2$. Hence, bronchoconstriction, vasodilation and mucus production are the components of early response. The late response occurs hours later and involves cytokines (IL-4, IL-5, IL-16 and TNF-$\alpha$) and eosinophilic chemotactic factor (ECF) and neutrophilic chemotactic factor. These mediators help endothelial cell adhesion and recruit neutrophils and eosinophils. These recruited cells release toxic enzymes, oxygen radicals and cytokines leading to occlusion of the bronchial lumen with mucus, proteins and cellular debris.

The subsequent sequelae are sloughing of the epithelium, thickening of the basement membrane, further increase in edema and hypertrophy of the bronchial musculature. ECF released from mast cells. Various lymphokines released at the site (IL-3, IL-5 and GM-CSF) contribute to the growth and differentiation of eosinophils. Eosinophils have receptors for Fc portion of IgE. When the target organism coated with IgE comes in contact with the Fc receptor on eosinophil, activation of eosinophil leads to degranulation and release of inflammatory mediators including leukotrienes, major basic protein, platelet activation factor, cationic protein and eosinophil-derived neurotoxins. These mediators may play a protective role, in parasitic infection by causing pores on the surface, leading to death of parasites (*Trichinella spiralis, Schistosoma*, filarial worms, etc.).

### *Detection of Type 1 Hypersensitivity*

Type 1 hypersensitivity is diagnosed by simple skin testing. A small quantity of potential allergen injected intradermally on forearm or back of the individual. If the person is allergic to allergen, there will be degranulation of the local mast cells releasing histamine and other mediators. In positive cases, there will be wheel and flare within 30 minutes. The advantage of the skin testing is that, it is inexpensive and large number of allergens can be tested at a time.

Another way in assessing the type 1 hypersensitivity is measuring IgE level by radioimmunosorbent test (RIST) and also by radioallergosorbent test (RAST) that detects the serum level of IgE specific for particular allergen.

### *Hyposensitization*

Repeated injections of increasing doses of allergens has been also known to reduce the severity of type 1 reaction.

### *Food Allergies*

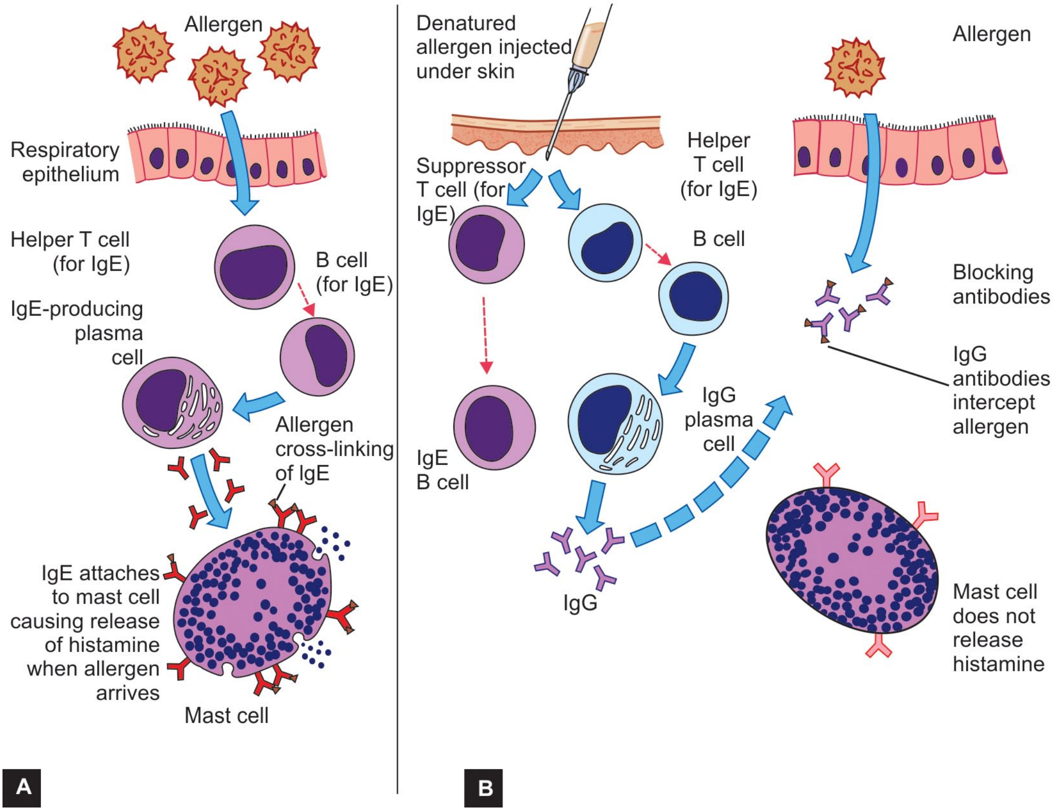
Certain foods can cause localized anaphylaxis. Allergen cross-linking of IgE on mast cells in the upper or lower gastrointestinal tract lead to the mediators release causing contraction of the smooth muscle of the intestine and has vomiting and diarrhea. The mast cell degranulation along the gut increase the permeability of the gut wall, permitting the allergens to enter the blood. Various symptoms can occur depending upon the site of deposition of the allergens. Therefore some individuals, after taking certain food suffer from asthmatic attack; where as others develop atopic urticaria, commonly known as hives, when a food allergen is carried to sensitized mast cells on the skin.

### *Atopic Dermatitis (Allergic Eczema)*

Atopic dermatitis is frequently associated with a family history of atopy and found in young children. Often, there is a high-level of serum IgE. The allergic individual develops skin eruptions that are erythematous and filled with pus. The skin lesions in allergic eczema, unlike in delayed hypersensitive reaction, which involve $T_h1$ cells, will have $T_h2$ cells and increase number of eosinophils.

### *Late-phase Reactions*

The late-phase reactions, distinct from late response found in asthma, develops 4 to 6 hours after initial type 1 reaction and persists for 1 to 2 days. The reaction is characterized by infiltration of neutrophils, eosinophils, macrophages, lymphocytes and basophils. The late-phase reaction is mediated partly by cytokines, released from mast cells (TNF-α and IL-1).


Allergen
Respiratory epithelium
Helper T cell (for IgE)
B cell (for IgE)
IgE-producing plasma cell
Allergen cross-linking of IgE
IgE attaches to mast cell causing release of histamine when allergen arrives
Mast cell
A
Denatured allergen injected under skin
Suppressor T cell (for IgE)
Helper T cell (for IgE)
B cell
IgE B cell
IgG plasma cell
IgG
Allergen
Blocking antibodies
IgG antibodies intercept allergen
Mast cell does not release histamine
B

**Figs 11.5A and B:** A proposed mechanism of action for desensitization allergy shots (hyposensitivity). **A.** In a normal allergic response, natural exposure to an allergen causes helper T cell to stimulate those B cells that mature into plasma cell to make IgE antibodies. After binding to mast cells, a second allergen exposure causes degranulation; **B.** Desensitization involves the injection of denatured allergen. Such shots may lead to tolerance, preventing B cells from maturing into plasma cells to make IgE antibodies. Exposure to the allergen also may activate those B cells that mature into plasma cells to make IgG (blocking) antibody. Such IgG antibodies can bind to incoming allergen before it reaches the IgE molecules attached to mast cells. Complexing the allergen with these attached IgE molecules would cause the mast cells to degranulate and release histamine, so blocking this step is the key to preventing allergic responses.

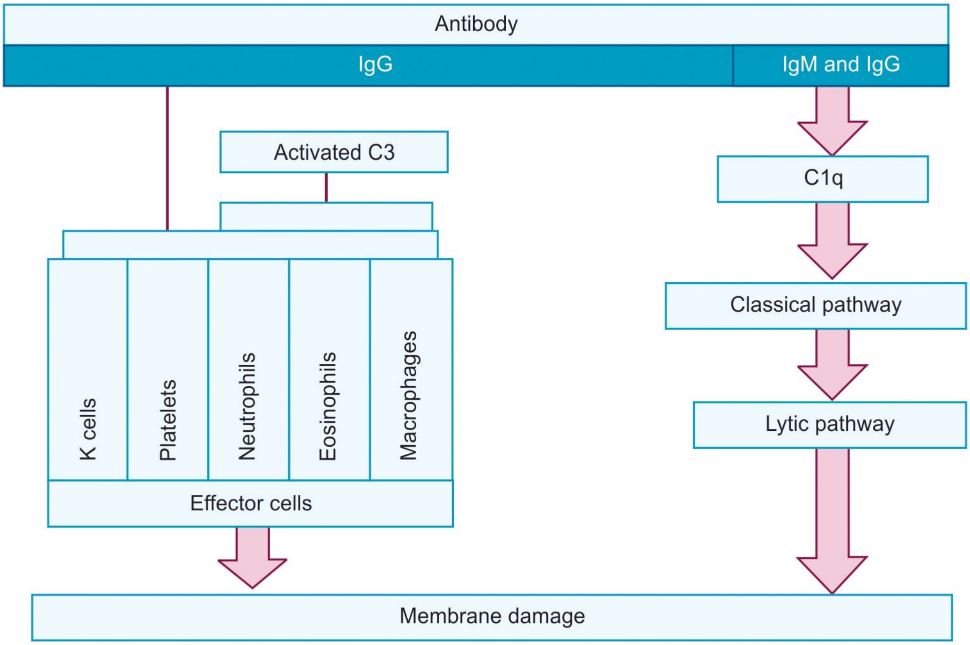


**Fig. 11.6:** Antibody-dependent cytotoxicity. Effector cells—K cells, platelets, neutrophils, eosinophils and cells of the mononuclear phagocytes series all have receptors for Fc, which they use to engage antibody bound to target tissues. Activation of complement C3 can generate complete-mediated lytic damage to target cells directly and also allows phagocytic cells to bind to their targets via C3b, C3bi or C3d, which also activate the cells.

Eosinophils play an important role in late-phase reaction. Eosinophils are attracted to the site of action in a good number of individuals suffering from allergic rhinitis. Repeated injections of increasing dose of allergen cause a shift to IgG antibodies, which block the allergen binding to the sensitized mast cell (Figs 11.5A and B) or may induce T cell-mediated suppression by a shift of $T_h2$ subset to $T_h1$ and IFN-α, which turns of the production of IgE. In this situation, The IgG is termed as blocking antibodies, because it competes with the allergen binds to it and form a complex, which can be phagocytosed by phagocytic cells.

## TYPE 2 HYPERSENSITIVITY REACTION (ANTIBODY-DEPENDENT CYTOTOXICITY)

Antibodies directed against cell surface antigen, promote cell death not only by complement-mediated lysis, but also by opsonization leading to phagocytosis or through non-phagocytic extracellular killing by certain lymphoid and myeloid cells (Fig. 11.6).

Type 2 hypersensitivity reactions involve antibody-mediated destruction of the cells. Antibody can mediate cell destruction by activating complement system and ultimately, producing pores by membrane attack complex. Antibody can also mediate cell destruc-

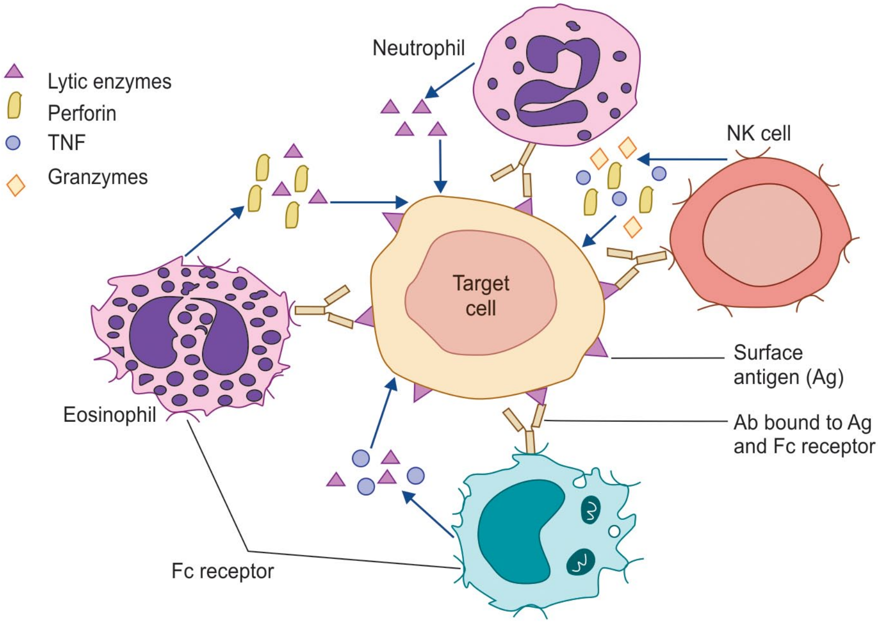


**Fig. 11.7:** Antibody-dependent cell-mediated cytotoxicity (ADCC). Non-specific cytotoxic cell are directed to specific target cells by binding to the Fc region of antibody bound to surface antigens on the target cells. Various substances (e.g. lytic enzymes, TNF, perforin, granzymes) secreted by the non-specific cytotoxic cells then mediate target cell destruction.

tion by antibody-dependent cell- mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc receptors bind to Fc region of antibodies on target cell and promote killing of the cells (Fig. 11.7).

Antibody bound to foreign cells may also serve as opsonin and facilitate phagocytosis (Figs 11.8A and B).

## Examples of Type 2 Reactions

### *Mismatched Transfusion Reactions*

Normal human red blood cells (RBCs) have genetically determined surface antigens (blood group system) that form the basis of different blood groups. Transfusion reaction can occur, when matching antigens and antibodies are present in the patient's blood. Such reactions can be triggered by any of the blood group antigen. If a sensitized patient received RBCs with a different blood cell antigen, IgM antibodies cause a hypersensitivity reaction against foreign antigen. The foreign red cells are agglutinated; complement is activated leading to hemolysis (Fig. 11.9).

### *Hemolytic Disease of the Newborn*

When a sensitized Rh-negative mother carries a second or subsequent Rh-positive fetus, the mother's anti-Rh antibodies cross the placenta and cause a type 2 hypersensitivity reaction in the fetus (Figs 11.10A to D).

### *Drug-induced Hemolytic Anemia and Purpura*

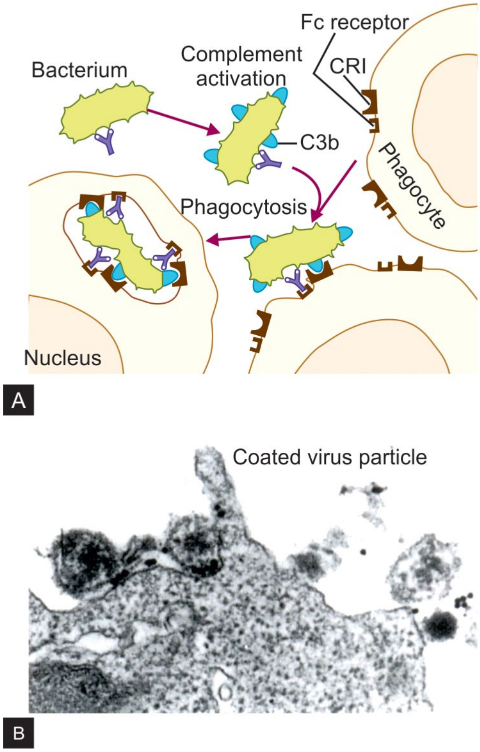
Certain antibiotics (penicillin, cephalosporin and streptomycin) can adsorb non-specifically to proteins on RBC and forms a protein carrier complex. In some individuals, this complex induces antibody formation causing hemolysis. Similar mechanism holds in causing sedormid purpura, when sedormid is given.

### *Goodpasture's Syndrome*

Autoantibodies specific for some basement membranes, such as antigens bind to basement membranes of the kidney glomeruli and the alveoli of the lungs. Subsequent complement activation leads to direct cellular damage, because of ensuing inflammatory response. Damage to glomerular and alveolar basement membrane leads to progressive kidney damage and pulmonary hemorrhage. The tissue damage is due to the type 2 hypersensitivity reaction.

### *Myasthenia Gravis*

Myasthenia gravis is formation of antibodies against acetylcholine receptors present in the motor end-plates of muscle, which blocks the normal binding of acetylcholine and also induces complement-mediated degradation of the receptors, resulting in progressive weakness of the muscle (Fig. 11.11).


Fc receptor
Complement activation
Bacterium
CRI
C3b
Phagocyte
Phagocytosis
Nucleus
A
Coated virus particle
B

**Figs 11.8A and B:** Role of complement and antibody in opsonization of microorganisms. **A.** Schematic representation of the role of C3b and antibody in opsonization; **B.** Electron micrograph of Epstein-Barr virus coated with antibody and C3b and bound to the Fc and C3b receptor (Cr1) on a B lymphocyte.

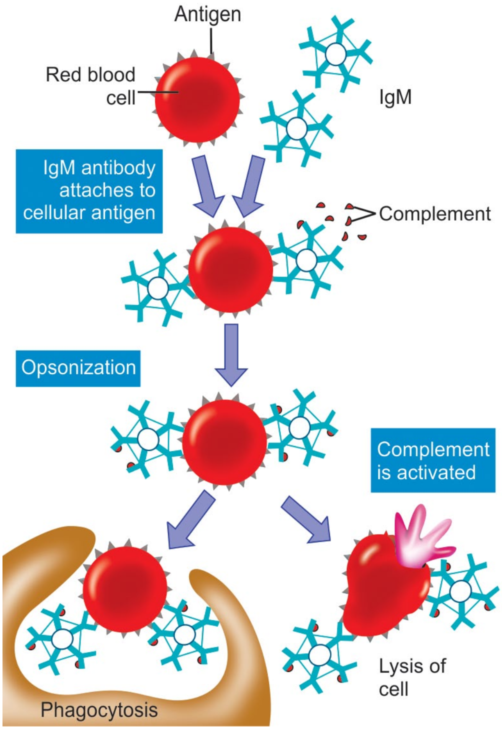
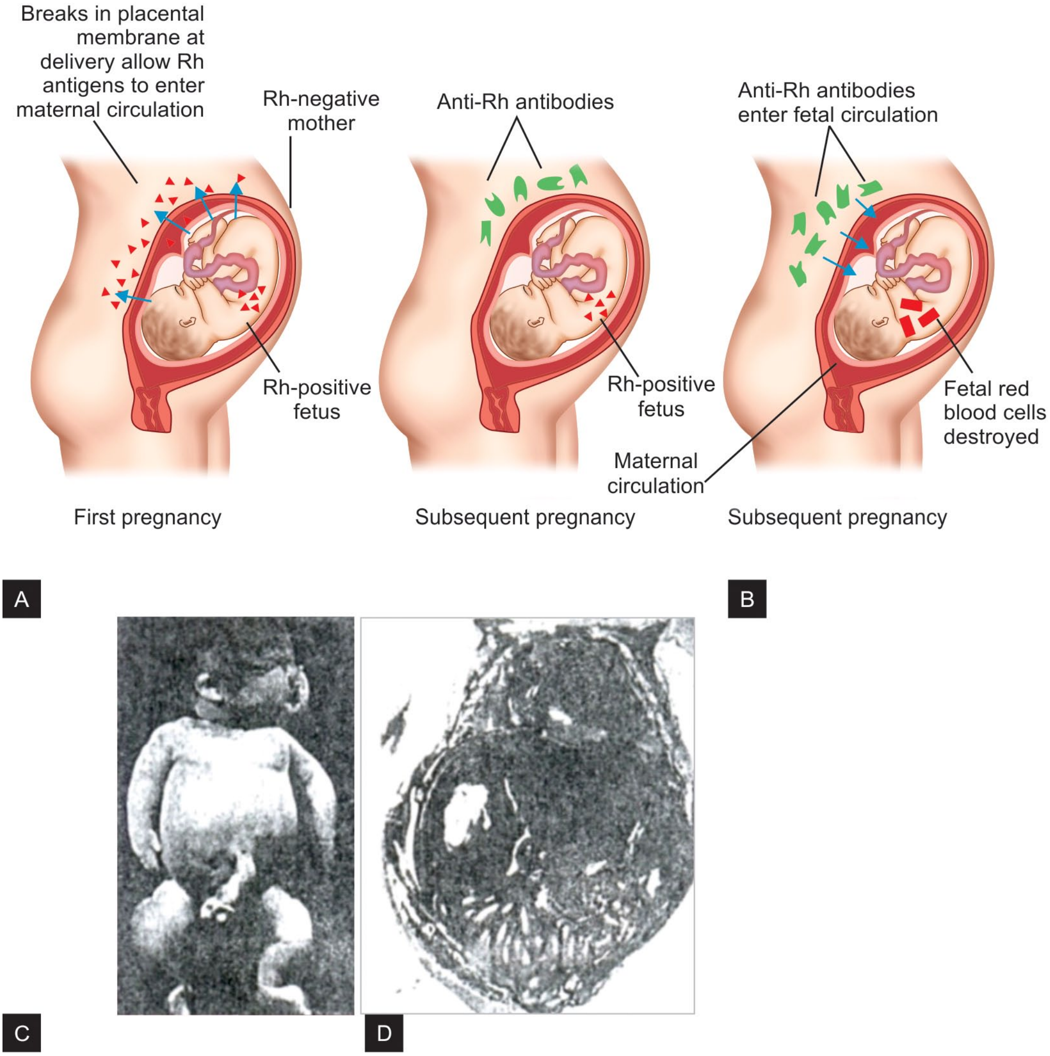

Antigen
Red blood cell
IgM
IgM antibody attaches to cellular antigen
Complement
Opsonization
Complement is activated
Lysis of cell
Phagocytosis

**Fig. 11.9:** The mechanism of cytotoxic (type 2) hypersensitivity. Mismatched red blood cell antigen usually is bound to IgM. Complement is activated and results in either subsequent phagocytosis or lysis of the red blood cells.


Breaks in placental membrane at delivery allow Rh antigens to enter maternal circulation
Rh-negative mother
Rh-positive fetus
First pregnancy
Anti-Rh antibodies
Rh-positive fetus
Maternal circulation
Subsequent pregnancy
Anti-Rh antibodies enter fetal circulation
Fetal red blood cells destroyed
Subsequent pregnancy
A
B
C
D

**Figs 11.10A to D:** Cause and effect of hemolytic disease of the newborn. **A.** The stage is set for an Rh-incompatibility when the mother is Rh negative and the fetus is Rh positive (which is usually the case if the father is Rh positive); **B.** Rh antigens may cross the placenta. Even if antibody production is not stimulated until delivery, the resulting antibodies will persist in the mother's circulation and attack the red blood cells of any subsequent Rh positive fetus. To prevent this situation Rhogam (anti-Rh antibody) is injected into the mother early in the pregnancy, immediately after delivery and in cases of miscarriage or abortion. Rhogam reduces exposure to the antigen and thus lessens anti-Rh antibody production; **C.** Child affected by hemolytic disease caused by Rh incompatibility; **D.** The liver is greatly enlarged.

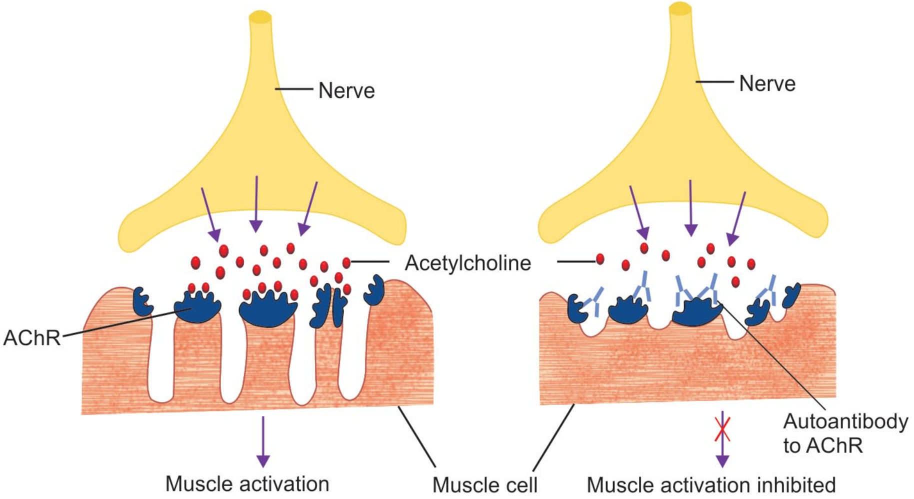


**Fig. 11.11:** Blocking autoantibodies in myasthenia gravis. Binding of autoantibodies to the acetylcholine receptors (right) block the normal binding of acetylcholine (burgundy dots) and subsequent muscle activation (left). In addition, the anti-AChR autoantibodies activate complements, which damage the muscle endplate; the number of acetylcholine receptors decline as the disease progresses. (AChR, acetylcholine receptor).

### *Graves' Disease*

Graves' disease is production of autoantibodies against thyroid-stimulating hormone (TSH) receptor. The antibodies induce or stimulate unregulated activation of thyroid hormones. The autoantibodies, so produced against TSH receptors are called long-acting thyroid stimulator (LATS), which is IgG in nature and can pass through the placental barrier and cause Graves' disease in neonates (Fig. 11.12).

## TYPE 3 HYPERSENSITIVITY REACTION (IMMUNE COMPLEX-MEDIATED)

Antigen and antibody complex exert a toxic effect, when they become localized in and around the blood vessels and initiate a damaging inflammatory response, as a result of activation of complement and infiltration of neutrophils.

These antigen-antibody complexes are deposited in and around blood vessels of joints, kidney, heart and skin leading to arthritis, nephritis, carditis, vasculitis respectively. These diseases caused by these complexes are collectively called immune complex diseases.

### Generalized Type 3 Reactions (Serum Sickness)

When large quantities of antigens enter into the bloodstreams and bind to antibody, immune complexes are formed. When the antigen is in excess, small complexes are formed, which are not cleared by phagocytic cells. They circulate and are deposited in and around blood vessels and cause tissue damage by type 3 reactions. Historically, generalized type 3 reactions were observed as a sequel to the administration of large quantities of horse antitoxic serum used to provide passive immunity in the treatment of diphtheria and tetanus. The condition is

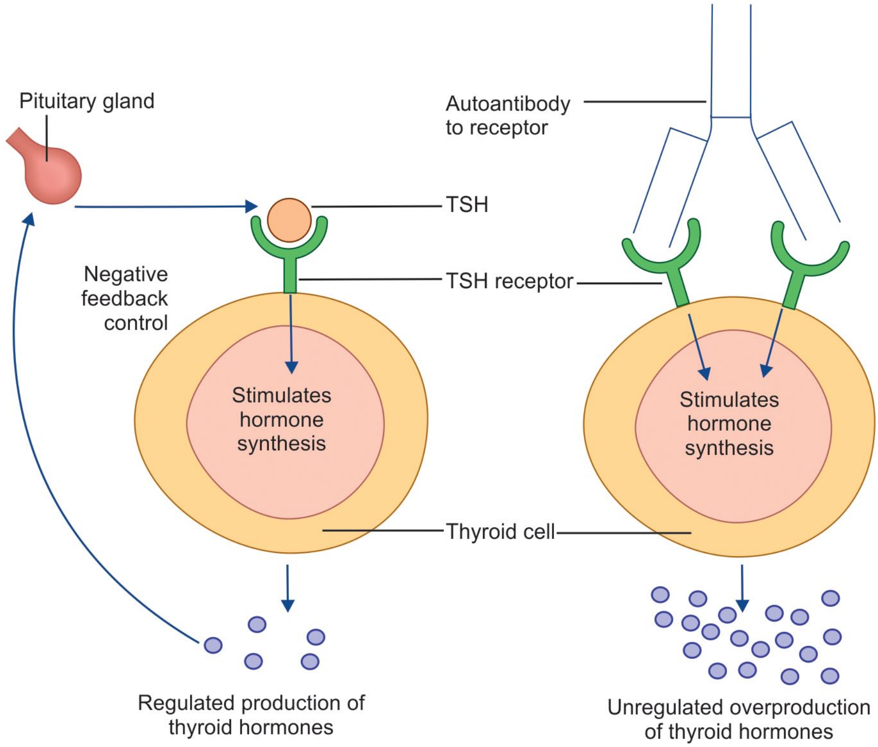

Pituitary gland
Autoantibody to receptor
TSH
Negative feedback control
TSH receptor
Stimulates hormone synthesis
Stimulates hormone synthesis
Thyroid cell
Regulated production of thyroid hormones
Unregulated overproduction of thyroid hormones

**Fig. 11.12**: Stimulating autoantibodies (Graves' disease). In Graves' disease, binding of autoantibodies to the receptors by thyroid-stimulating hormone (TSH) induces unregulated activation of the thyroid, leading to overproduction of the thyroid hormones (purple dots).

unrelated to antitoxin content and can be produced in response to normal horse serum protein or other serum containing foreign protein. Foreign serum proteins are antigens and cause antibody formation. These complexes circulate for several days and give rise to inflammatory lesions of serum sickness, which include:

1. Painful swelling of joints (arthritis).
2. Transient albuminuria and renal lesion (glomerulonephritis).
3. Carditis.
4. Skin lesions (vasculitis).

There may be also fever, urticarial rashes, abdominal pain, nausea and vomiting.

The immune complexes activate the complement system's array of immune effector molecules (Fig. 11.13). The C3a, C4a, C5a complement split products are anaphylatoxins that cause localized mast cell's degranulation and release of vasoactive amines (including histamine and 5-HT). Direct interaction of immune complexes with basophils and platelets (via Fc receptors) also induce the release of vasoactive amines. The ultimate consequence is the increase in local vascular permeability. C3a, C5a and C567 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune complex deposition.

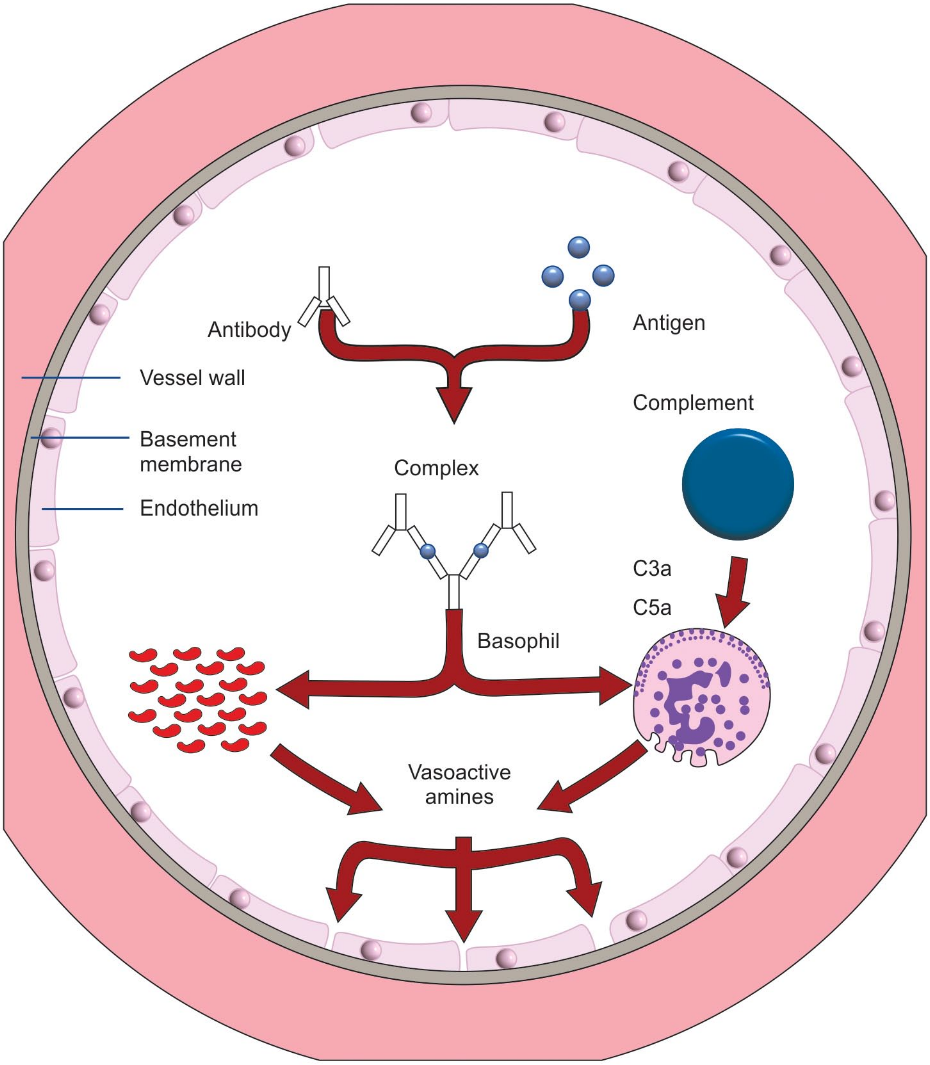

Antibody
Antigen
Vessel wall
Basement membrane
Endothelium
Complement
Complex
C3a
C5a
Basophil
Vasoactive amines

**Fig. 11.13:** Immune complexes act on complement to generate C3a and C5a, which in turn stimulate basophils to release vasoactive amines. The complex also act directly on basophils and platelets (in humans) to release vasoactive amine. The amines released (e.g. histamine, 5-hydroxytryptamine) cause endothelial cell retraction and thus increase vascular permeability.

Much of the tissue damage in type 3 reactions caused by the lytic enzymes by neutrophils, as they attempt to phagocytose immune complexes. A neutrophil binds to C3b-coated immune complex by means of specific complement receptor for C3b. Because the immune complex is deposited on the basement membrane, phagocytosis cannot occur. The frustrated neutrophils being unable to phagocytose

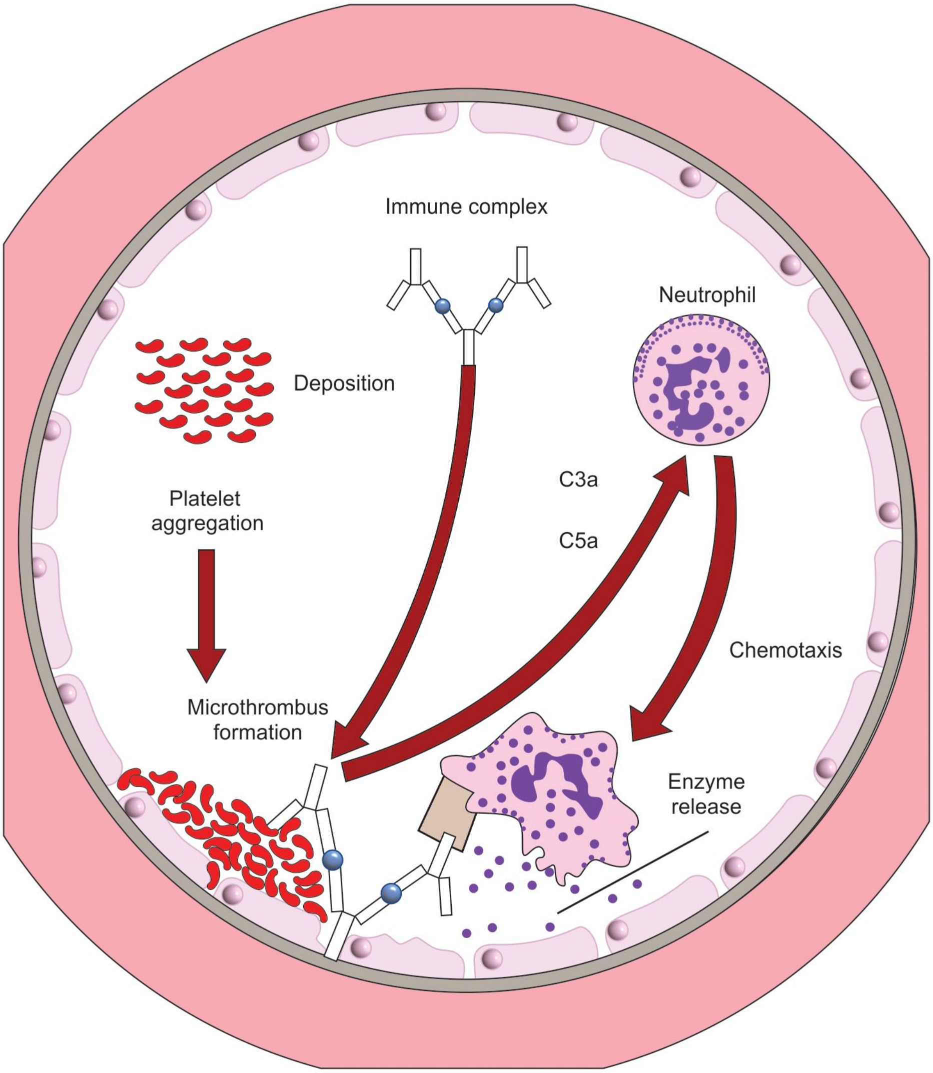

Immune complex
Neutrophil
Deposition
Platelet aggregation
C3a
C5a
Chemotaxis
Microthrombus formation
Enzyme release

**Fig. 11.14:** Increased vascular permeability allows immune complexes to be deposited in the blood vessel wall. This induces platelet aggregation and complement activation. The aggregated platelets form microthrombi on the exposed collagen of the basement membrane of the endothelium. Neutrophils are attracted to the site by complement products, but cannot ingest the complexes. Therefore they exocytose their lysosomal enzymes, causing further damage to the vessel wall.

vomit out the lytic enzymes. Further activation of the membrane attack mechanism of the complement system can also contribute to the destruction of the tissue. In addition, the activation of complement can induce platelet aggregation and the release of clotting factors causing formation of microthrombi (Fig. 11.14).

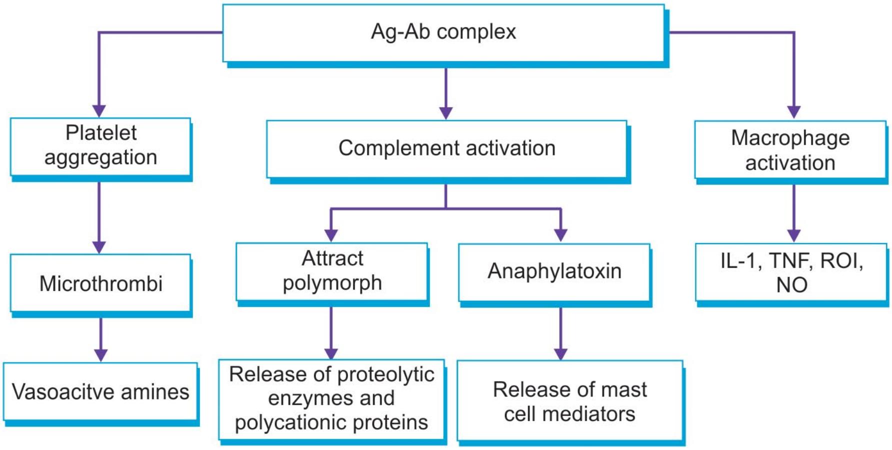

Ag-Ab complex
Platelet aggregation
Complement activation
Macrophage activation
Microthrombi
Attract polymorph
Anaphylatoxin
IL-1, TNF, ROI, NO
Vasoacitve amines
Release of proteolytic enzymes and polycationic proteins
Release of mast cell mediators

**Fig. 11.15:** Flowchart showing mechanism of hypersensitivity type 3 reaction

Macrophages are stimulated to release cytokines particularly TNF-α and IL-1, which also add to the tissue damage by causing inflammation (Fig. 11.15).

### *Mechanism of Type 3 Reaction*

Formation of circulating immune complexes contribute to the pathogenesis of several conditions other than serum sickness.

*Autoimmune diseases:* They are systemic lupus erythematosus (SLE) rheumatoid arthritis.

*Drug's reaction:* There is allergy to penicillin and sulfonamide.

*Infectious diseases:* These are poststreptococcal glomerulonephritis, lepromatous leprosy [erythema nodosum leprosum (ENL)] during drug therapy, nephrotic syndrome (due to *Plasmodium malariae* in children), viral hepatitis, meningitis, infectious mononucleosis trypanosomiasis, subacute sclerosing panencephalitis (SSPE).

## Localized Type 3 Reaction (Arthus Reactions)

A different method of inducing local immune complex inflammations is by injection of the antigen into the skin of an animal, which has a high circulating level of antibody against antigen. A hemorrhagic edematous reaction occurs in the skin, which take 2 to 8 hours to develop and persist for 12 to 24 hours or result in tissue necrosis. The necrosis is due to the destruction of small blood vessels by thrombi. This is the Arthus reaction named after its discoverer (Fig. 11.16).

Intrapulmonary Arthus type reactions induced by bacterial spores, fungi or dried fecal proteins can cause pneumonitis or alveolitis. These reactions are known by a variety of common names reflecting the source of antigen. Some of the examples are:

- Farmer's lung disease: Moldy hay containing spores of thermophilic actinomycetes
- Pigeon fancier's disease: Antigen is protein present in dry faces of pigeon
- Cheese-washer's disease: The antigen is penicillium casei spore
- Furrier's lung disease: Antigen is a fox fur protein.

## Detection of Immune Complex

Deposited immune complex can be visualized using immunofluorescence.

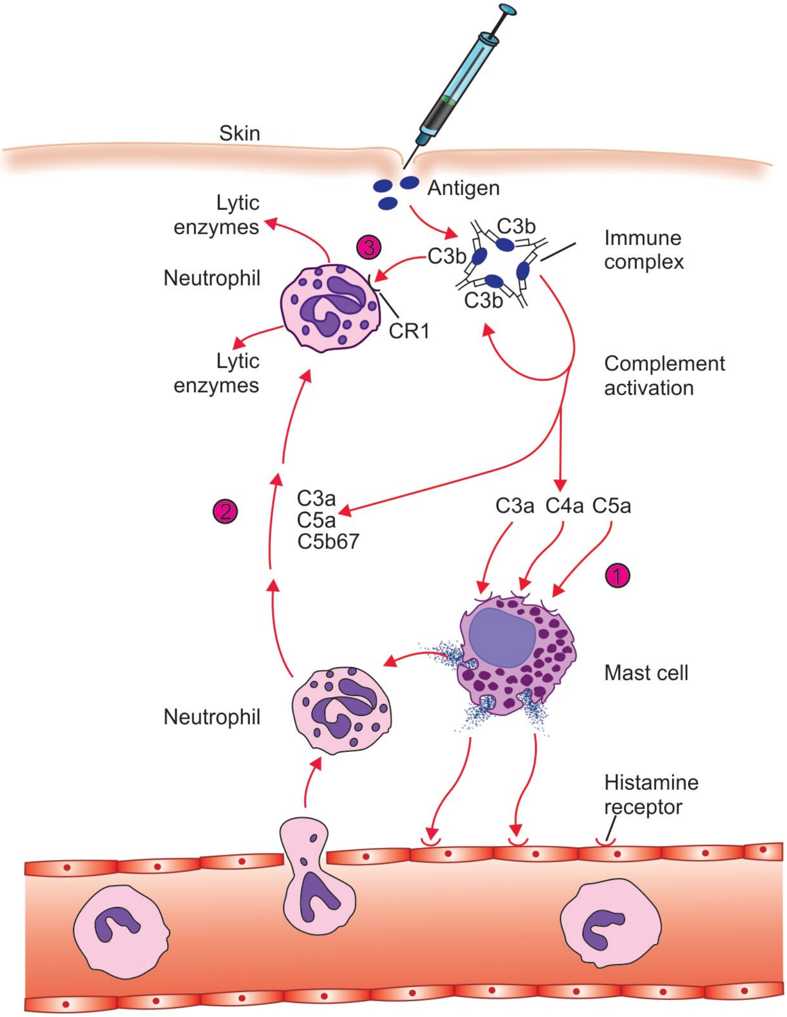

Skin
Antigen
Lytic enzymes
③
C3b
C3b
C3b
Immune complex
Neutrophil
CR1
Lytic enzymes
Complement activation
②
C3a
C5a
C5b67
C3a C4a C5a
①
Mast cell
Neutrophil
Histamine receptor

**Fig. 11.16:** Development of a localized Arthus reaction (type 3 hypersensitive reaction). Complement activation initiated by immune complexes (classical pathway) produces complement intermediates that; **1.** Mediate mast cell degranulation; **2.** Chemotactically attract neutrophils; **3.** Stimulate release of lytic enzymes from neutrophils trying to phagocytose C3b-coated immune complexes.

Precipitation of the immune complex with polyethylene glycol (PEG) and estimation of precipitated IgG is frequently used to identify high molecular IgG.

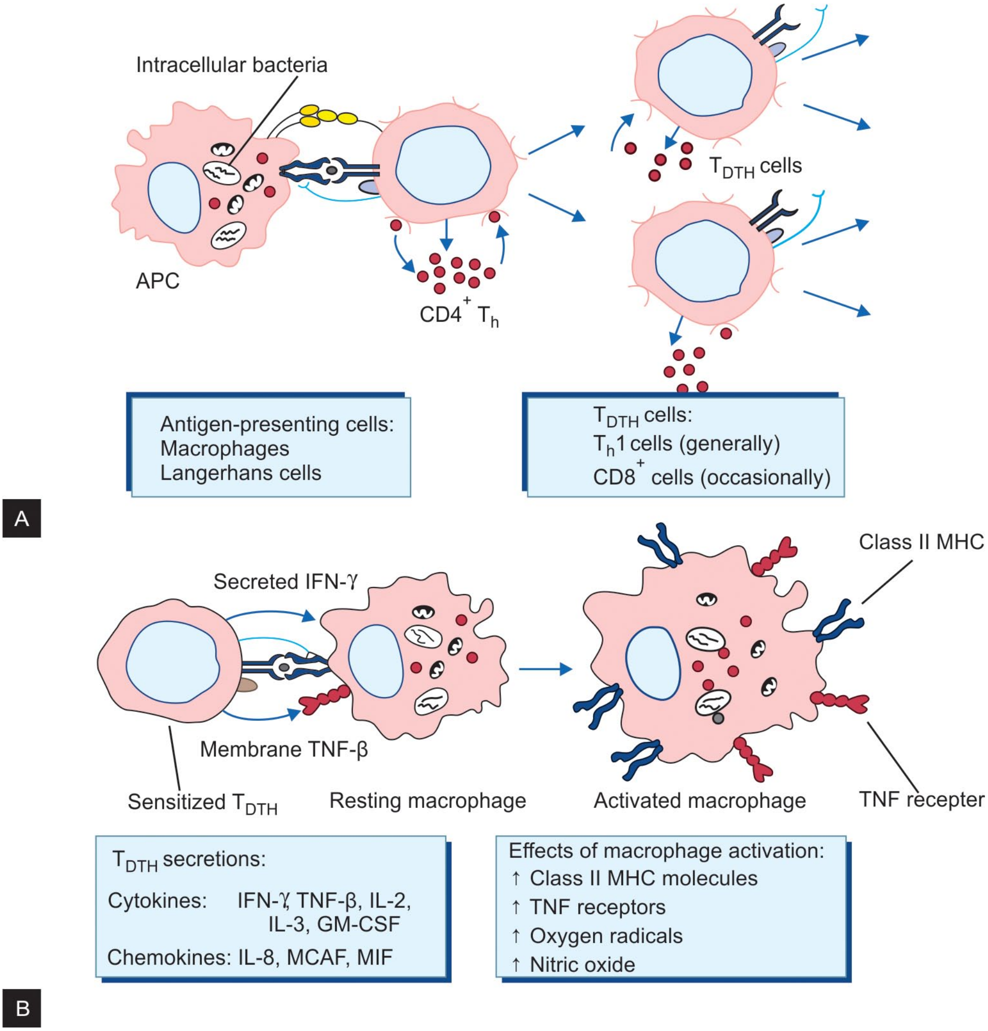
Circulating complexes are often identified by their affinity for complement C1q either radiolabeled C1q or solid-phase C1q.

## TYPE 4 CELL-MEDIATED (DELAYED) HYPERSENSITIVITY

Type 4 cell-mediated hypersensitivity is also called delayed hypersensitivity (DH), because the reactions take more than 12 hours. The reactions are mediated by T helper cells specifically $T_h1$ cells without the participation of antibodies. Sometimes, cytotoxic T cells (Tc) are also involved.

## Mechanism of Delayed Hypersensitivity

Activation of $T_h1$ cells (Fig. 11.17A) by antigen on appropriate antigen-presenting cells, results in the secretion of various cytokines including IL-2, IFN-γ, macrophage inhibiting factor (MIF) and TNF-β. The overall effects of these cytokines is to draw macrophages to the area and to activate them promoting increased phagocytic activities and increased concentration of lytic enzymes for effective killing. The release of lytic enzymes into the surroundings lead to tissue destruction. These reactions typically take 98 to 72 hours to develop. As opposed to neutrophils found


Intracellular bacteria
APC
$CD4^+$ $T_h$
$T_{DTH}$ cells
Antigen-presenting cells:
Macrophages
Langerhans cells
$T_{DTH}$ cells:
$T_h1$ cells (generally)
$CD8^+$ cells (occasionally)
A
Secreted IFN-γ
Class II MHC
Membrane TNF-β
Sensitized $T_{DTH}$
Resting macrophage
Activated macrophage
TNF recepter
$T_{DTH}$ secretions:
Cytokines: IFN-γ, TNF-β, IL-2, IL-3, GM-CSF
Chemokines: IL-8, MCAF, MIF
Effects of macrophage activation:
↑ Class II MHC molecules
↑ TNF receptors
↑ Oxygen radicals
↑ Nitric oxide
B

**Figs 11.17A and B:** Mechanism of delayed hypersensitivity. **A.** Sensitization phase; **B.** Effector phase.

in type 3 reaction, macrophages are the major components of the infiltrate (Fig. 11.17B).

## Examples of DH Disorders

There are three common examples of DH. They are contact dermatitis, tuberculin hypersensitivity and granulomatous hypersensitivity.

### *Contact Dermatitis*

Delayed hypersensitivity, sometimes, result from skin contact with a variety of chemicals such as metals (nickel, chromium, etc.), dyes (picryl chloride, dinitrochlorobenzene, etc.), drugs (penicillin, etc.), formaldehyde, turpentine, cosmetics,

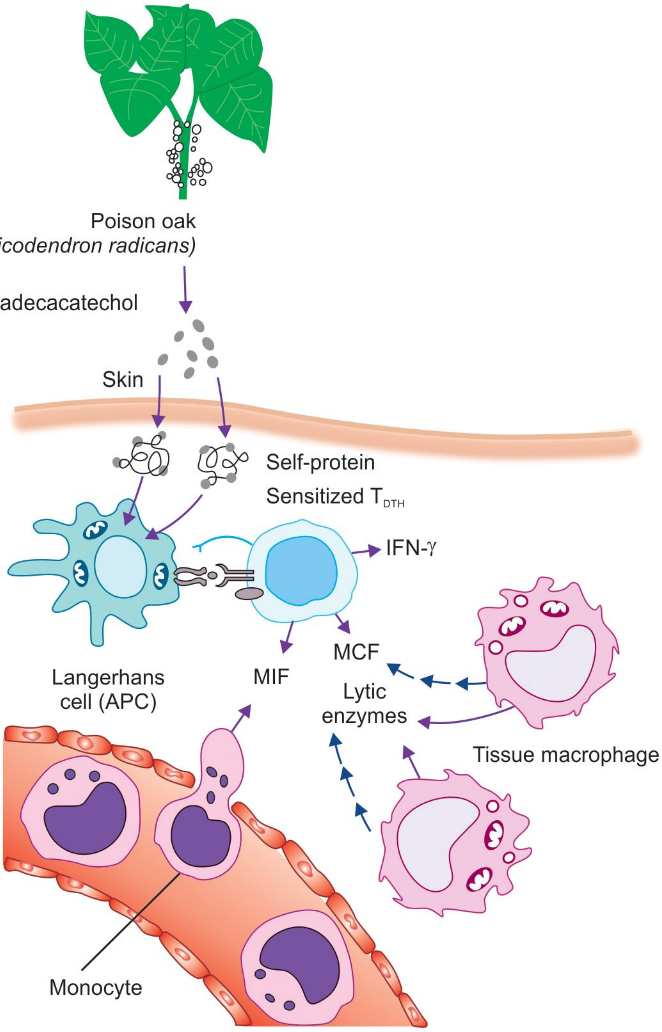

Poison oak
*(Toxicodendron radicans)*
Pentadecacatechol
Skin
Self-protein
Sensitized $T_{DTH}$
IFN-γ
MCF
Lytic enzymes
MIF
Langerhans cell (APC)
Tissue macrophage
Monocyte

**Fig. 11.18:** Development of delayed-type hypersensitivity reaction after a second exposure to poison oak. Cytokines such as IFN-γ, MCF and MIF released from sensitized $T_{DTH}$ cells mediate this reaction. Tissue damage results from lytic enzymes released from activated macrophages (MCF, macrophage chemotactic factor; MIF, migration inhibition factor).

poison oak and poison ivy (Fig. 11.18). Sensitization is particularly liable, when contact is with an inflamed area of the skin and the chemicals applied in a oilybase. The substances themselves are not antigenic, but may acquire antigenicity in combination with skin protein.

Molecules too small to cause immune reactions pass through the skin, where they become antigens by binding to normal proteins on Langerhans cells of the epidermis. These cells, which carry MHC II molecules migrate to lymph nodes, where they act as antigen-presenting cells to $T_h1$ cells. Within 4 to 8 hours after the next exposure, a hypersensitive reaction begins and eczema occurs within 48 hours.

### *Tuberculin Hypersensitivity*

When a small dose of tuberculin or purified protein derivative (PPD) is injected intradermally in an individual sensitized to tuberculin protein by prior infection or immunization, an indurated inflammatory reaction occurs at the site within 48 to 72 hours. Similar antigens from the bacterium that causes leprosy (*Mycobacterium leprae*) and protozoa that causes leishmaniasis (*Leishmania tropica*) produce similar reactions in sensitized individuals. The antigen activates $T_h1$ cells, which in turn produce cytokines that attract large number of lymphocytes, monocytes and macrophages to infiltrate dermis. The normally soft tissue of the dermis becomes raised, hard red region called induration.

### *Granulomatous Hypersensitivity*

Granulomatous type of hypersensitivity occurs when macrophages have engulfed pathogens, but have failed to kill them. Inside the macrophage, the protected pathogens survive and sometimes continue to multiply. $T_h1$ cells sensitized to the antigen

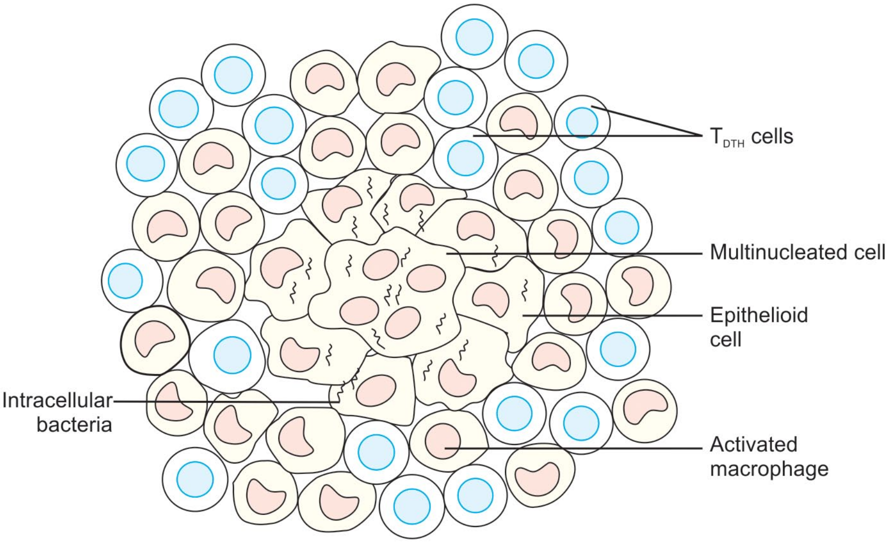
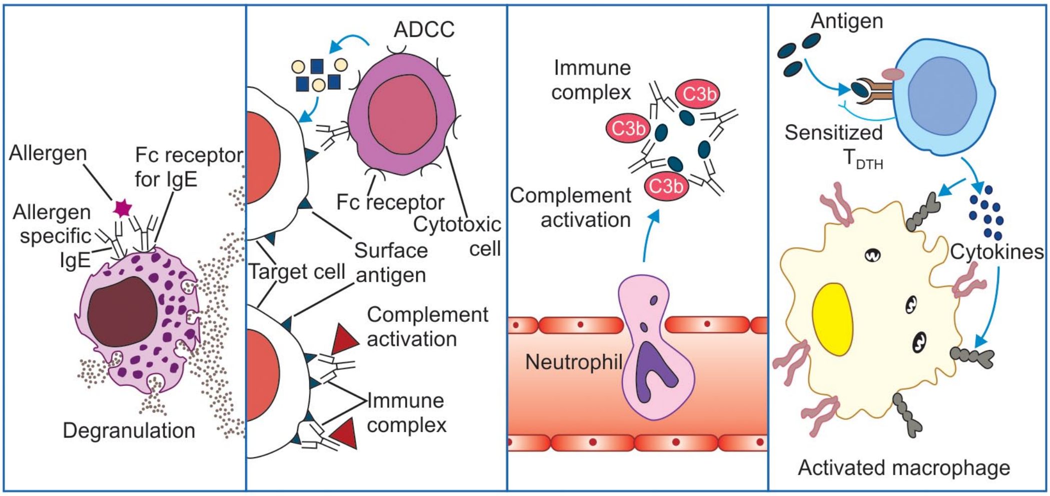


**Fig. 11.19:** A prolonged DTH response can lead to formation of a granuloma, a nodule-like mass. Lytic enzymes released from activated macrophages in a granuloma can cause extensive tissue damage.

of the pathogen elicit the hypersensitivity reaction attracting several cell types to the skin (leproma) or lung (tubercle) (Fig. 11.19). This kind of hypersensitivity is the most delayed of all (Fig. 11.20), appearing 4 weeks or more after the exposure of the antigen. Such persistent and continuous antigenic stimuli are also typical of the bacterial disease, listeriosis and many fungal and helminthic infections (Table 11.2).


Allergen
Fc receptor for IgE
Allergen specific IgE
Degranulation
ADCC
Fc receptor
Cytotoxic cell
Surface antigen
Target cell
Complement activation
Immune complex
Immune complex
C3b
C3b
C3b
Complement activation
Neutrophil
Antigen
Sensitized $T_{DTH}$
Cytokines
Activated macrophage

| Type I | Type 2 | Type 3 | Type 4 |
|---|---|---|---|
| Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators. | Ab directed against cell surface antigens, mediates cell destruction via complement activation or ADCC. | Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils. | Sensitized $T_{DTH}$ cells release cytokines that activate macrophages or Tc cells, which mediate direct cellular damage. |
| Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies and eczema. | Typical manifestations include blood transfusion reactions, erythroblastosis fetalis and autoimmune hemolytic anemia. | Typical manifestations include localized Arthus reactions and generalized reactions such as serum sickness, necrotizing vasculitis, glomerular nephritis, rheumatoid arthritis and systemic lupus erythematosus. | Typical manifestations include contact dermatitis, tubercular lesions and graft rejection. |

**Fig. 11.20:** The four types of hypersensitive responses

### *T Cell-mediated Cytotoxicity*

In some instances, CD8 T lymphocytes may be responsible for causing type 4 hypersensitivity reactions. Reactive chemical agents (haptens) pass through the cell membrane and bind to cytoplasmic proteins to produce neoantigens. Peptides derived from these haptenated proteins are presented along with MHC class I molecules to sensitize and elicit cytotoxic T lymphocyte (CTL) response.

**Table 11.2** Intracellular pathogens that induce delayed type (type 4) hypersensitivity

| **Intracellular bacteria** | **Intracellular viruses** |
|---|---|
| *Mycobacterium tuberculosis*<br>*Mycobacterium leprae*<br>*Listeria monocytogenes*<br>*Brucella abortus* | Herpes simplex virus<br>Variola (smallpox)<br>Measles virus |
| **Intracellular fungi** | **Intracellular parasites** |
| *Pneumocystis jiroveci*<br>*Candida albicans*<br>*Histoplasma capsulatum*<br>*Cryptococcus neoformans* | *Leishmania species* |

## STUDY QUESTIONS

### Essay Questions

1. Define and classify hypersensitivity. Discuss the mechanism of anaphylaxis.
2. What is hypersensitivity? Explain the mechanism of cytotoxic hypersensitivity reaction with examples.
3. Mention the Coombs and Gell classification of hypersensitivity. What triggers an immune complex hypersensitivity reaction and what are its effects?
4. What is an Arthus reaction? Give some examples of intrapulmonary Arthus reaction.
5. Explain the mechanism of cell-mediated hypersensitivity. Why it is called delayed hypersensitivity?

### Short Notes

1. Atopy.
2. Arthus reaction.
3. Type 2 hypersensitivity.
4. Type 3 hypersensitivity.

## SUGGESTED READING

1. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. Nat Genet. 1997;15:389.
2. Biron CA. Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. Semin Immunol. 1998; 10:383.
3. Black JG. Microbiology: Principles and Applications, 3rd edition. USE: Printice Hall College div; 1996.
4. Borish L. Genetics of allergy and asthma. Ann. Allergy Asthma Immunol. 1999; 82:413.
5. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
6. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
7. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
8. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
9. Male David, Brostoff Jonathan, Roitt Ivan, et al. Immunology, 7th edition. Mosby, Elsevier; 2006.
10. Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical book); 2007.
11. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
12. Tortora, Funke, Case. Microbiology: An Introduction, 6th edition; 1998.

# Autoimmunity

# 12

Immune system, under normal circumstances does not react against self-antigens. Paul Ehrlich realized that the immune system can behave abnormally attacking on self-antigens. He termed this condition as 'horror autotoxicus'. It is now clear that though mechanisms of self-tolerance normally protect an individual from self-reactive lymphocytes, there are evasions of self-tolerance. They result in an inappropriate response of immune system against self-components and that is termed as 'autoimmunity'.

Earlier, it was believed that all self-reactive lymphocytes were eliminated during development and failure to eliminate these lymphocytes, completely lead to autoimmunity. Towards the end of 1970, it was established that not all the self-reactive lymphocytes are deleted during T and B cell maturation. The presence of these self-reactive lymphocytes in normal individuals can cause autoimmune reactions, as they are regulated through clonal anergy or clonal suppression. A breakdown in this regulation can lead to activation in self-reactive clones of T or B cells, mounting humoral or cell-mediated immune response against self-antigens.

Sometimes, the damage to self-cell is caused by antibodies and sometimes by T cell. For example, in autoimmune hemolytic anemia, the destruction of cell is caused by autoantibodies to red cells. Autoantibodies to thyroglobulin is the cause of tissue destruction in Hashimoto's thyroiditis. Insulin-dependent diabetes and multiple sclerosis are primarily due to the action of self-reactive T cells.

## PROBABLE MECHANISMS OF AUTOIMMUNITY

Several mechanisms play important role in causing autoimmunity. Evidences suggest that autoimmunity does not develop from a single event, but rather from a number of different events. There is difference in susceptibility to autoimmunity between the two sexes. Women are more prone to thyroiditis, systemic lupus erythematosus (SLE), multiple sclerosis (MS) and rheumatoid arthritis (RA). The preferential susceptibility may be due to hormonal differences between sexes and the potential effect of fetal cells in the natural circulation during pregnancy.

### Genetic Factors

Genetic factor predisposes autoimmunity. It has been seen that children of parent, who has autoantibody against one organ, develop autoantibody to same or different organs. The strongest association between an human leukocyte antigen (HLA) allele and autoimmunity is seen in ankylosing spondylitis, an inflammatory disease of vertebral joints. Individuals, who have HLA-B27 are 90 times more prone to suffer from ankylosing spondylitis than the individuals with other HLA-B allele (Table 12.1).

**Table 12.1** Major histocompatibility complex (MHC) association with autoimmune diseases

| Human leukocyte antigen genes | Disease |
|---|---|
| *B8* | Myasthenia gravis |
| *B27* | Acute uveitis<br>Ankylosing spondylitis<br>Reiter's disease |
| *CW6* | Psoriasis vulgaris |
| *DR2* | Goodpasture's syndrome<br>Multiple sclerosis |
| *DR3* | Grave's disease<br>Multiple sclerosis<br>Myasthenia gravis<br>Systemic lupus erythematosus |
| *DR4* | Pemphigus vulgaris<br>Rheumatoid arthritis |
| *DR3/DR4* | Type1 insulin-dependent diabetes mellitus |
| *DR5* | Hashimoto's thyroiditis |

## Association with T Cell Receptor

The presence of T cell receptors containing particular variable alpha (Vα) and variable beta (Vβ) domains has also been linked to a number of autoimmune diseases including experimental autoimmune encephalitis and its human counter part, MS.

## Release of Sequestered Antigens

If clonal deletion fails to remove the self-reactive T helper ($T_h$) cells, autoimmunity develops. If they survive and proliferate, they can attack self-anigens and trigger B cell activity with antibody production. Antigens hidden in the tissues and lacking contact with B or T cells during immune system development or clonal deletion could be released through physical injury. For example, sperms arise late in the development and are sequestered from circulation. However during vasectomy, some of the spermatozoa are released into the circulation and induce autoantibody formation. Similarly, the release of lens protein after eye damage or of heart muscle antigens after myocardial infarction (MI) has been shown to lead autoantibody formation.

There are certain sites in the body, which remain isolated from the immune system. They are called immunologically privileged sites. In addition to the lumen of the testicular tubule, these sites include the cornea and the anterior chamber of the eye, the brain and the uterine environment during pregnancy. The avascularity of cornea and the fluid-filled anterior chamber of the eye may help to protect the delicate structures of eye from the damage and permanent injury that could follow inconsequence to strong inflammatory response. In addition, the cells in the anterior chamber of the eye widely express the Fas ligand (CD178), which binds to Fas (CD95) on the activated T cells, which undergo apoptotic death (Fig. 12.1). Thus, the anterior chamber can be protected by killing autoreactive 'T cells'. The brain is protected by a mechanism called blood-brain barrier (BBB). The BBB consists of tightly packed vascular endothelium, which limits the flow of cells and various antigens from the vasculature to the brain, thus reducing the immunological reactions in the brain. Because of three-dimensional configuration, a part of a molecule (epitope) may remain in the interior to avoid contact with immune system. This is known as cryptic epitope. The presence of rheumatoid factor, associated with rheumatoid inflammatory disease serves as example for this phenomenon (Fig. 12.2). Epitope binding conformationally changes the antibody [immunoglobulin G (IgG)] fragment crystal-

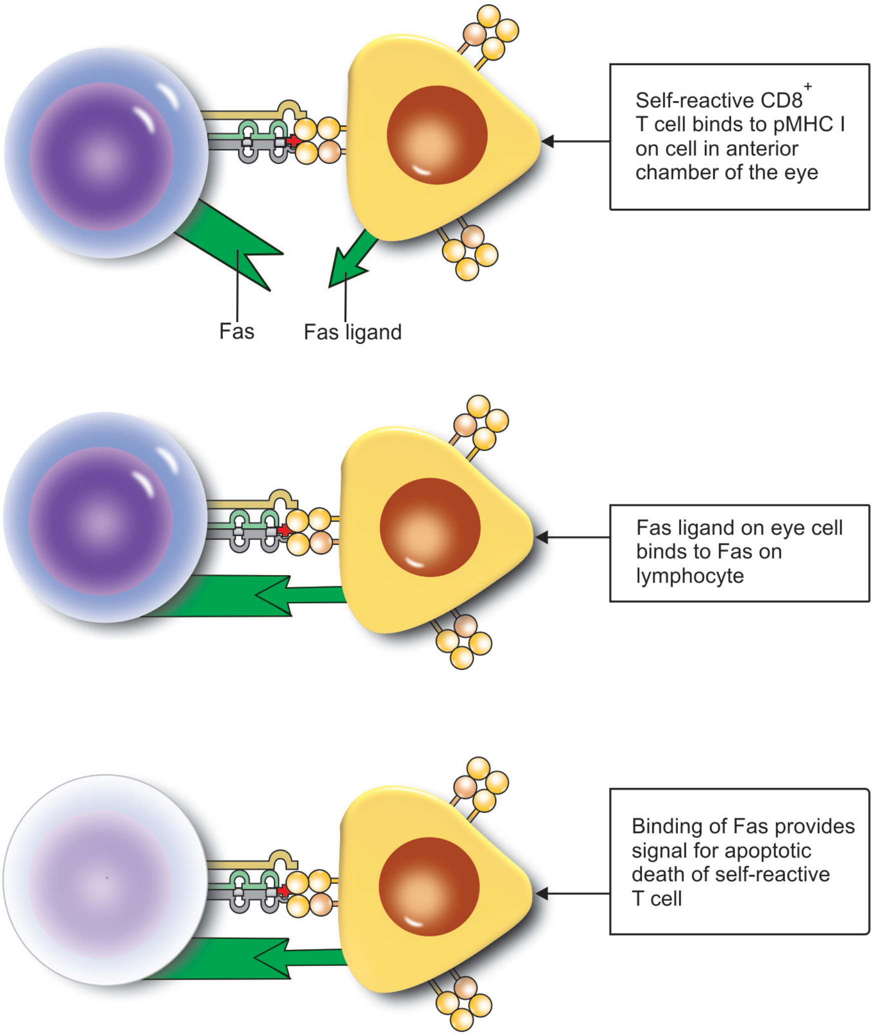

Self-reactive CD8+ T cell binds to pMHC I on cell in anterior chamber of the eye
Fas
Fas ligand
Fas ligand on eye cell binds to Fas on lymphocyte
Binding of Fas provides signal for apoptotic death of self-reactive T cell

**Fig. 12.1:** Role of Fas ligand in protection of cells within immunologically privileged sites. Fas ligand is widely expressed on cells in the anterior chamber of the eye. When autoimmune T cells attempt to bind to cells of the anterior chamber, Fas ligand binds to Fas molecules expressed by T cells. This binding induces apoptotic death of the Fas-bearing cell (in this case, the T cell) and immune-mediated damage to the cells of the anterior chamber is avoided.

lizable (Fc) region, exposing cryptic carbohydrate structures. IgM antibodies are formed against the newly exposed carbohydrate structures. IgM antibodies directed at the cryptic carbohydrate structures on antigen-bound IgG molecules are called rheumatoid factors.

## Antigenic or Molecular Mimicry

A number of viruses and bacteria have been shown to possess antigenic determinants that are identical or similar to normal host cell components. Such molecular similarity appears in a wide variety of organisms.

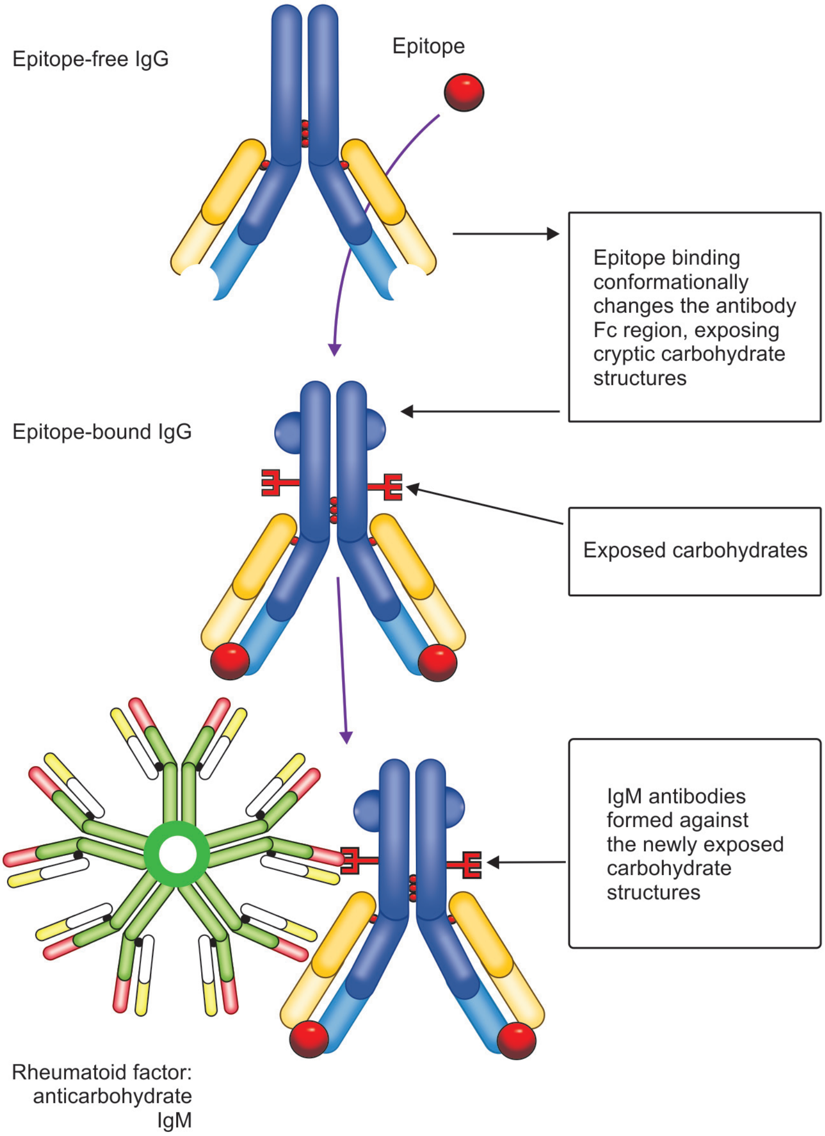

Epitope-free IgG
Epitope
Epitope binding conformationally changes the antibody Fc region, exposing cryptic carbohydrate structures
Epitope-bound IgG
Exposed carbohydrates
IgM antibodies formed against the newly exposed carbohydrate structures
Rheumatoid factor: anticarbohydrate IgM

**Fig. 12.2:** Rheumatoid factor (IgM produced against IgG) results from recognition of cryptic epitopes. Binding of antibodies, including IgG, to their epitopes produce a conformational change in the Fc region, exposing sites that become available for the binding of complement and recognition by cellular Fc receptors. The exposed sites include previously cryptic carbohydrate structures that, once available, can be recognized and bound by IgM molecules.

Molecular mimicry has been suggested as one of the mechanism that leads to autoimmunity. One of the best examples of this type of autoimmune reaction is postrabies encephalitis in the person, who had received encephalitis neural antirabies vaccine. The rabies vaccine is prepared by culturing the virus in rabbit's brain. Hence, the vaccine contains rabbit brain cell antigens. These rabbit brain cells could induce formation of antibodies and activate T cells, which could cross-reacts with recipient's own brain cells, due to organ specificity, leading to encephalitis. Cross-reacting antibodies are also thought to be the cause of heart damage in the rheumatic fever, which can sometimes develop after *Streptococcus pyogenes* infection. Antigenic similarity exists between group A β-hemolytic *S. pyogenes* (M proteins) with the molecules found on the valves and membranes of the heart. If there is sufficient rise of IgG and IgM against 'M' protein, they may bind to the host tissue and cause cardiac damage (Fig. 12.3).

There are several examples of autoimmune diseases, which are associated with infectious organisms. A number of reactive arthritis occurs more frequently in persons, who suffered from food poisoning. Ankylosing spondylitis and Reiter's diseases (affecting joints of lower limb and the gastrointestinal/genital/urinary tracts) have increased frequencies in individuals, who carry the *HLA-B27* gene and have been infected by *Klebsiella*. Subsequently, it has been found out that some structural similarities exist between HLA-B27 molecule and certain proteins expressed by *Klebsiella*. The acetylcholine receptor, which is the target of autoimmune myasthenia gravis shares some structural resemblance with some poliovirus proteins. Molecular mimicry appears to be involved in several autoimmune diseases including diabetes. Certain peptide fragments of coxsackievirus and cytomegalovirus (CMV) cross-react with glutamate decarboxylase, a major target of autoreactive T cells found in patients with type 1 diabetes.

## Epitope Spreading

Epitope spreading is a phenomenon that may contribute to the influence of infectious organism on autoimmunity. In addition to the epitope that initiates a response leading to autoimmunity, certain epitopes are developed later during the pathogenesis of the disease. For example, initial responses against an infectious agent may result in the damage that exposes self-epitopes in ways that subsequently trigger the true autoimmune responses. Further, the dominant self-epitope targeted by an autoimmune response does not remain constant over the course of the disease. Thus, the epitopes those are involved in the pathogenesis may be different from the epitopes, which initiated the immune response. This complicates attempts to device therapy. In autoimmune diseases such as SLE, inflammatory bowel disease (Crohn's disease and ulcerative colitis), multiple sclerosis, pemphigus vulgaris, etc. epitope spreading plays an important role.

## Inappropriate Expression of Class II MHC Molecule

In individuals with insulin-dependent diabetes and Graves' disease there is more expression of major histocompatibility complex (MHC I) and MHC II molecules in beta cells of islets of the Langerhans and acinar cells of thyroid than the normal individuals. Normally these cells contain only MHC I molecules, but not MHC II molecules. This inappropriate expression of MHC II molecules may serve to sensitize $T_h$ cells to peptides, derived from the beta cells of the pancreas or acinar cells of the thyroid, allowing activation of B cells or $T_h$ cells or T cytotoxic ($T_C$ cells) against self-antigen. It was hypothesized that trauma or viral infection may induce localized inflammatory response and thus increase concentrations of interferon-gamma (IFN-γ). IFN-γ induces high level of MHC II molecules in non-antigen presenting cells.

## Mutation

Mutation might give rise to aberrant proteins to which B cells react producing plasma cells that make autoantibodies.

### *Polyclonal Cell Activation*

A number of viruses and bacteria can induce polyclonal activation of B cell, non-specifically. Viruses such as Epstein-Barr virus (EBV),

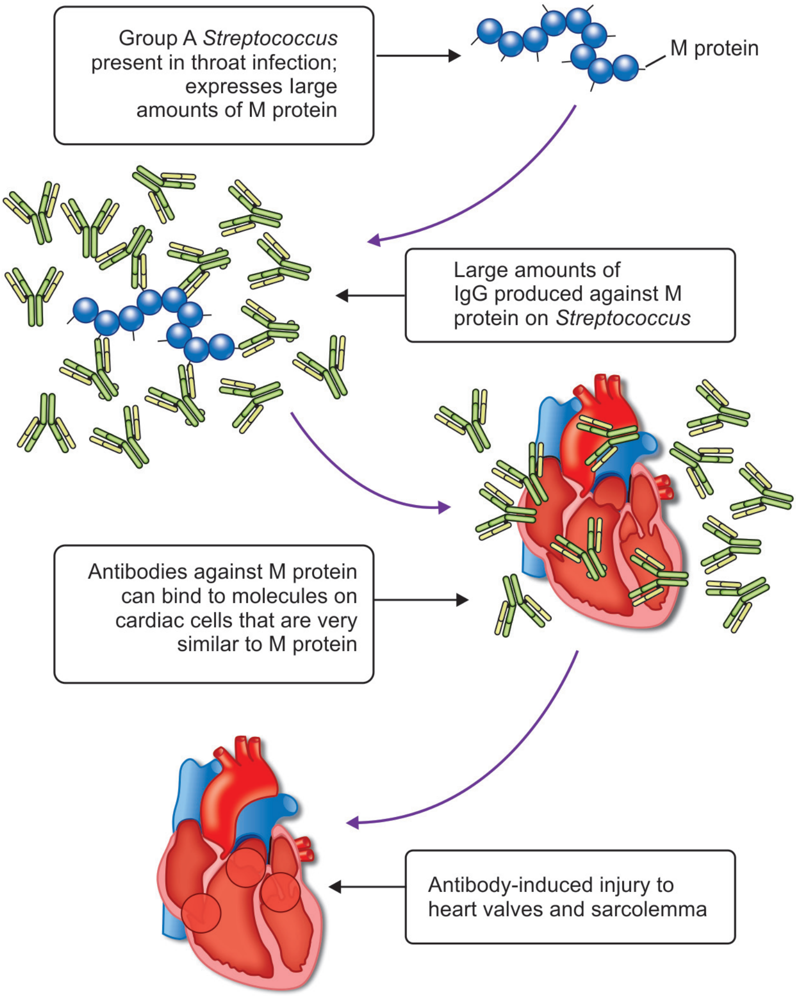

Group A *Streptococcus* present in throat infection; expresses large amounts of M protein
M protein
Large amounts of IgG produced against M protein on *Streptococcus*
Antibodies against M protein can bind to molecules on cardiac cells that are very similar to M protein
Antibody-induced injury to heart valves and sarcolemma

**Fig. 12.3:** Association of autoimmune diseases with serial responses to different epitopes. Some autoimmune diseases have alternating periods of exacerbation and remission of clinical signs (relapsing-remitting pattern). In some models of human autoimmune disease, the relapsing phases of exacerbation have been shown to be due to a series of newly generated responses to different epitopes.

CMV, human immunodeficiency virus (HIV) and gram-negative bacteria are polyclonal activators and lead to proliferation of clones that express IgM without the help of $T_h$ cells. If self-reactive B cells are activated, antibodies to self-antigens are produced.

## LOSS OF SUPPRESSION

Suppressor cells serve to maintain immune tolerance. As the age advances, the number of these suppressor cells decline. The risk of autoimmune disease is enhanced in aged individuals permitting the previously suppressed autoreactive lymphocytes to become active. A pattern of increasing risk with increasing age is commonly seen in autoimmune diseases such as SLE.

## AUTOIMMUNE DISEASES

Autoimmune diseases fall into two groups: organ-specific and non-organ-specific disease. In organ-specific autoimmune diseases, the immune response is directed against the components of one organ or gland, so that the effects are largely confined to that organ only. The cells are damaged by humoral immunity or cell-mediated immunity or by both.

In non-organ-specific (systemic) autoimmune diseases, the response is directed to a broad range of antigens existing in different organs and tissues. The damage is extensive, which involves both cell-mediated and antibody-mediated immune response, such as immune complex deposit (Table 12.2).

### Examples of Organ-specific Diseases

#### *Hashimoto's Thyroiditis*

Hashimoto's thyroiditis is seen, most frequently in middle-aged women. The individual produces autoantibodies and sensitized $T_h$ cells specific for thyroid antigens. The delayed-type hypersensitivity (DHT) response leads to infiltration of lymphocytes and plasma cells. The inflammatory response causes goiter or visible enlargement of thyroid gland. Autoantibodies are formed against a number of thyroid proteins including thyroglobulin and thyroid peroxidase. Binding of autoantibodies to these substances interfere in iodine uptake, causing decreased production of thyroid hormones leading to hypothyroidism.

#### *Autoimmune Anemia*

Autoimmune anemia includes autoimmune hemolytic anemia; drug-induced hemolytic anemia and pernicious anemia.

In autoimmune hemolytic anemia, red cell membrane-bound autoantibodies trigger complement-mediated lysis or antibody-mediated opsonization followed by phagocytosis. Coombs' test is used for diagnosis of autoimmune hemolytic anemia in which the red cells are incubated with an antihuman IgG antiserum. If IgG autoantibodies are present on the red cells, the cells are agglutinated by the antiserum.

In drug-induced hemolytic anemia, drugs such as penicillin, methyldopa, etc. bring about a change in the red cell's antigenicity causing hemolysis in the same mechanism, as that of autoimmune hemolytic anemia.

In pernicious anemia, autoantibodies are formed against membrane-bound protein on the parietal cells (intrinsic factor), which block the intrinsic factor-mediated vitamin B12 absorption leading to pernicious anemia.

#### *Insulin-dependent Diabetes Mellitus*

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune reaction of pancreas, which involves the beta cells of the islets of Langerhans against which there is formation of autoantibodies. The autoimmune attack destroys the beta cells resulting to a decreased production of insulin and consequently increased level of blood glucose. Au-

**Table 12.2** Spectrum of autoimmune diseases

| Disease | Self-antigens | Immune response |
|---|---|---|
| | **Organ-specific autoimmune disease** | |
| Addison’s disease | Adrenal cells | Autoantibodies |
| Autoimmune hemolytic anemia | RBC* membrane protein | Autoantibodies |
| Goodpasture’s syndrome | Renal and lung basement membrane | Autoantibodies |
| Graves’ disease | Thyroid-stimulating hormone receptor | Autoantibodies |
| Hashimoto’s thyroiditis | Thyroid protein and cells | $T_h$† (stimulating) cells and autoantibodies |
| Idiopathic thrombocytopenic purpura | Platelet membrane proteins | Autoantibodies |
| Insulin-dependent diabetes mellitus | Pancreatic beta cells | $T_h$ cells, autoantibodies |
| Myasthenia gravis | Acetylcholine receptors | Autoantibody (blocking) |
| Myocardial infarction | Heart | Autoantibody |
| Pernicious anemia | Gastric parietal cells, intrinsic factor | Autoantibody |
| Poststreptococcal | Kidney | Antigen-antibody complex |
| Spontaneous infertility | Sperm | Autoantibodies |
| | **Systemic autoimmune diseases** | |
| Ankylosing spondylitis | Vertebrae | Immune complex |
| Multiple sclerosis | Brain or white matter | $T_h$ and $T_c$‡ cells, autoantibodies |
| Rheumatoid arthritis | Connective tissue, IgG§ | Autoantibodies immune complex |
| Scleroderma | Nuclei, heart, lungs, GI‖ tract and kidney | Autoantibodies |
| Sjögren’s syndrome | Salivary gland, liver, kidney, thyroid | Autoantibodies |
| Systemic lupus erythematosus | DNA¶, nuclear protein, RBC and platelets | Autoantibodies immune complex |

*RBC, red blood cells; †$T_h$ cells, T helper cells; ‡$T_C$ cells, cytotoxic T cells; §IgG, immunoglobulin G; ||GI, gastrointestinal; ¶DNA, deoxyribonucleic acid.

toantibodies to beta cell may constitute cell destruction, facilitating either by antibody-complement cell lysis or by antibody-dependent cell-mediated cytotoxicity (ADCC). The beta cell destruction is accomplished by the action of cytotoxic T lymphocytes (CTL), the cytokines [tumor necrosis factor-α (TNF-α), gamma interferon (IFN-γ), interleukin-1 (IL-1)], released at the site and also by the cytolytic action of the enzymes liberated from the macrophages.

Following the destruction of islet beta cells, there is abnormality in glucose metabolism resulting in serious metabolic problems that include ketoacidosis and increased urine productions. Lately, atherosclerotic vascular lesions develop causing gangrene of the extremities, because of impede vascular flow. This may lead to renal failure and blindness.

### *Addison's Disease*
### *(Chronic Primary Hypoadrenalism)*

Addison's disease of the adrenal glands, either is caused by exogenous agent such as *Mycobacterium tuberculosis* or have an idiopathic cause, believed to have an immunologic mechanism. 50 percent of the patients have autoantibodies to the microsomes of the adrenal cells, as compared to 5 percent in the general population. Autoantibodies directed against adrenal cells are believed to play main role in the pathogenesis of Addison's disease.

The symptoms of Addison's disease include weakness, fatigue, anorexia, nausea, weight loss and diarrhea. There may be skin pigmentation, vascular collapse and hypotension. Finally, there is atrophy of the adrenal glands leading to the loss of function of adrenal cortex. The diagnosis is confirmed by demonstration of antiadrenal antibodies by indirect immunofluorescence test. Very often Addison's disease occurs in association with other autoimmune diseases such as Hashimoto's thyroiditis, pernicious anemia and diabetes mellitus.

Other organ-specific autoimmune diseases such as myasthenia gravis, Graves' disease and Goodpasture's syndrome have been discussed in previous chapter 11 (Hypersensitivity).

## Examples of Non-organ-specific Diseases

### *Rheumatoid Arthritis*

Rheumatoid arthritis affects mainly the joints of the hands and feet although it can extend to other tissues. It develops early in life (between the age of 30 and 40) and leads to crippling disabilities. It is 2 to 3 times common in women than in men.

It is characterized by inflammation and destruction of cartilage in the joints causing deformities. The cause is not known. Some believe that immune system recognizes self-antigen, as foreign antigen and induce reaction. Inflammation activates specific cells in the joint and attracts lymphocytes. $T_h1$ cells recognize a self-antigen in the joint and trigger the activation of B cells, which differentiate to plasma cells and secrete IgG antibody. $T_h1$ cells also release cytokines that trigger local phagocytes to release degrading enzymes from lysosomes. Within the inflamed synovia are B cells, plasma cells, $CD4^+$ T cells and various types of inflammatory cytokines such as TNF-$\alpha$, IL-1, IL-8 and IFN-$\gamma$. The binding of IgG molecules to unknown epitope triggers conformational changes in the Fc regions that expose 'hidden' sites, some of which facilitate the binding of complement or recognition by cellular Fc receptors and some of which expose cryptic carbohydrate structures that can be recognized and bound by IgM antibodies. IgM antibodies directed at the cryptic carbohydrate structures on antigen-bound IgG molecules are called rheumatoid factors. The binding of IgM to IgG augment the formation of immune complexes. In advanced stages of RA, deposition and complement fixation of these immune complexes may contribute not only to joint destruction, but also to vasculitis, carditis and pleuritis. RA is an example of autoimmune disease, which involves both humoral (type III hypersensitivity) and cell-mediated (type IV hypersensitivity) injury (Refer to Fig. 12.2).

### *Systemic Lupus Erythematosus*

The name is derived from the reddened skin rash (erythematous) that resembles, a wolf mask (lupus in Latin is wolf). The butterflyshaped rash appears over the nose and cheeks of about 30 percent of SLE patients (Fig. 12.4). SLE typically appears in women between 20 and 40 years of age; the ratio of female to male is 10:1. SLE is characterized by fever, skin rashes, arthritis, pleurisy and kidney dysfunction.

In SLE, autoantibodies (IgG, IgM and IgA) are formed primarily against the components of deoxyribonucleic acid (DNA), but also can be made against blood cells [red blood cells (RBCs), platelets and leukocytes], clotting factors, neurons and histones. Autoantibodies specific for RBCs and platelets, can lead to complement-mediated lysis resulting hemolytic anemia and thrombocytopenia respectively. When immune complexes of autoantibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III hypersensitivity reaction develops. The complexes activate complement cascade and generate membrane attack complexes (MAC) and complement split products, which damage the wall of the blood vessels leading to vasculitis and glomerulonephritis. Elevated serum levels of complement split product (C5a) induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. The number of circulating neutrophils decline (neutropenia) and occlusion of small blood vessels develop vasculitis. Widespread tissue damage ensue following occlusions. Most SLE patients eventually die from kidney failure, as glomeruli fail to remove the waste from the blood. SLE also affects the blood vessels of heart valves and joints.

Laboratory diagnosis of SLE consists of demonstrations of antinuclear antibodies against double-stranded or single-stranded DNA, histones, nucleoproteins and nuclear ribonucleic acid (RNA). Indirect immunofluorescent technique can be used to know the presence of autoantibodies in patient's serum by the demonstration of nuclear fluorescence.

### *Multiple Sclerosis*

Multiple sclerosis is one of the few common causes of neurological disabilities, prevalent in Western countries, more so in USA. The symptoms may vary from mild numbness in the limbs to paralysis or loss of vision. Most people are diagnosed between the ages of 20 to 40 years. The women are affected 2 or 3 times more than men. Like most of the autoimmune diseases, the cause is not well understood. Genetic influences are important contributing factors, as MS runs in family and found in siblings, twins. As some viruses cause demyelinating diseases, it is tempting to think that virus infection plays significant role in causation of MS.

Individuals with disease produce autoreactive T cells that take part in the formation of inflammatory lesions along the myelin sheath of nerve fibers. The cerebrospinal fluid (CSF) of patients of MS reveal sensitized T lymphocytes, which infiltrate the brain tissue and cause characteristic inflammatory lesions destroying the insular covering (myelin sheath) of the nerve fibers leading to a number of neurologic dysfunctions.

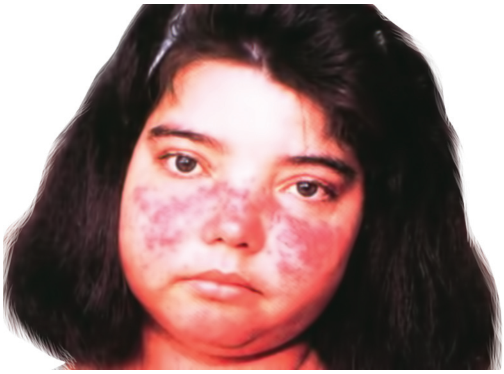


**Fig. 12.4:** Characteristic 'butterfly' rash over the cheeks of a young girl with systemic lupus erythematosus *(Courtesy:* Steinman L, 1993, Scientific American, 269(3):80).

## STUDY QUESTIONS

### Essay Questions

1. Define autoimmunity. Discuss the postulations that could explain autoimmune response.
2. What is autoimmunity? Discuss the organ-specific autoimmune diseases.

### Short Notes

1. Sequestrated antigen.
2. Molecular mimicry.
3. Epitope spreading.

## SUGGESTED READING

1. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). Nat Genet. 1997; 154:389-92.
2. Biron CA. Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. Semin Immunol. 1998; 10:383-90.
3. Black JG. Microbiology Principles and Applications, 3rd edition. USA: Printice Hall College div; 1996.
4. Borish L. Genetics of allergy and asthma. Ann Allergy Asthma Immunol. 1999;825: 413-24.
5. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
6. Goldsby RA, Kuby TJ. Immunology, 6th edition. 2007.
7. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
8. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: Mc Graw Hill, Lange Basic Science; 2010.
9. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby, Elsevier; 2006.
10. Stites. Basic and Clinical Immunology, 8th edition. 2007.
11. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology, 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
12. Tortora, Funke, Case. Microbiology: an introduction, 6th edition. 1998.

# Immunodeficiency Disorders

# 13

The immune system is not always perfect in performing its function smoothly. When the system over-reacts, it may lead to hypersensitivity. Similarly, when the system loses its sense of self and begins attacking host cells and tissues, the result is autoimmunity. When the system fails to protect the hosts from infectious agents or malignant cells, the result is immunodeficiency. The immunodeficiency may be the result of defective immunity, both innate and specific, because of genetic abnormality (primary) or there is a loss of function because of the damage by physical, chemical or biological agents (secondary). In primary immunodeficiency, the defect is at birth although it may not manifest itself until later in life. By far, the most common secondary immunodeficiency is the acquired immunodeficiency syndrome (AIDS), which results from the infection with human immunodeficiency virus (HIV) (Fig. 13.1).

## PRIMARY IMMUNODEFICIENCIES

A primary immunodeficiency may affect either innate or adaptive immunity. The defects, which arise in 'T' and 'B' lineage cells may be differentiated from the defects due to complement disorders or defect in the phagocytic property of macrophages and neutrophils. Immunodeficiencies are conveniently categorized by the type or developmental stage of the cells involved (Fig. 13.2).

Primary deficiencies in immunological function can arise through failure of any of the developmental processes from stem cell to functional end cell. Defects in the development of the common lymphoid stem cell give rise to severe combined immunodeficiency. Both T and B lymphocytes fail to develop, but functional phagocytes are present. The myeloid cell disorders affect phagocytic function. Most of the primary immunodeficiencies are inherited.

## DISORDERS OF SPECIFIC IMMUNITY

### B-cell Immunodeficiency Disorders

*X-linked Agammaglobulinemia (Bruton's Agammaglobulinemia)*

X-linked agammaglobulinemia (XLA) is the first immune deficiency disease to have been recognized. Very low levels of all types of immunoglobulins (IgG, IgA, IgM, IgD and IgE) are found and a virtual absence of B cells in young boys. Infants with this disorder usually become symptomatic following the natural loss of transplacentally acquired maternal IgG at about 5 to 6 months of age. They suffer from severe chronic bacterial infections, which can be controlled readily with gamma globulin and antibiotic treatment. Cell-mediated immunity (CMI) is not affected, allograft rejection is normal. The genetic defect has recently been defined as a deficiency of the

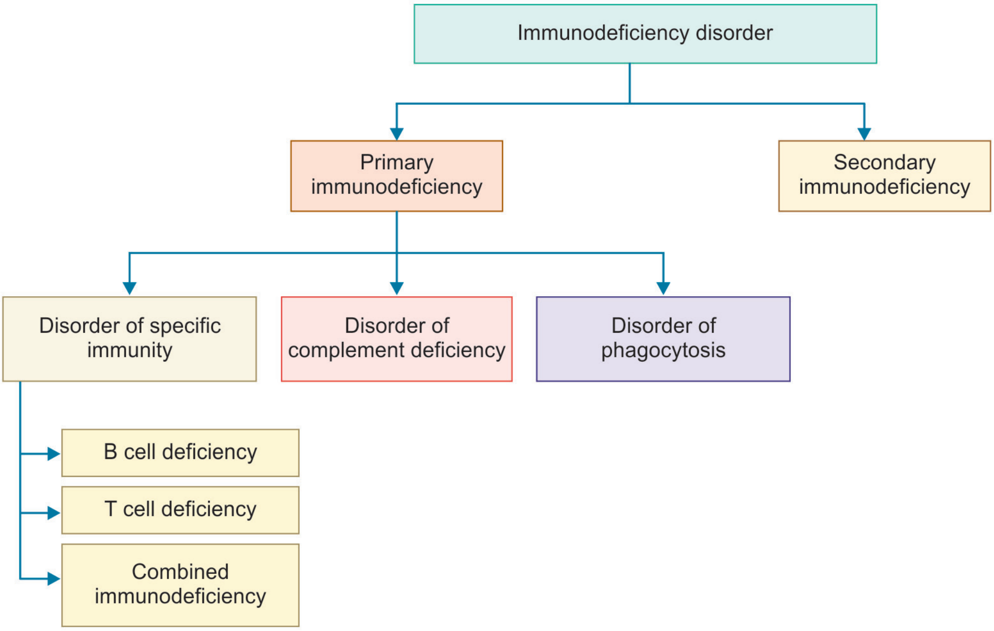

Immunodeficiency disorder
Primary immunodeficiency
Secondary immunodeficiency
Disorder of specific immunity
Disorder of complement deficiency
Disorder of phagocytosis
B cell deficiency
T cell deficiency
Combined immunodeficiency

**Fig. 13.1:** Flow chart showing types of immunodeficiency disorders

enzyme, B-cell progenitor kinase (BPK), a cytoplasmic tyrosine kinase. The gene encoding this enzyme is on the long arm of the chromosome at Xq22.

The most common organisms responsible for infection are *Streptococcus pneumoniae, Haemophilus influenzae* other streptococci, meningococci and *Pseudomonas*.

### *Transient hypogammaglobulinemia of infancy*

All infants develop physiologic hypogammaglobulinemia at approximately 5 to 6 months of age. At this time, maternal IgG is slowly catabolized, the infant begins synthesizing its own IgG by this age. Occasionally, an infant may fail to initiate IgG synthesis at this time, resulting in a prolonged period of hypogammaglobulinemia termed transient hypogammaglobulinemia of infancy (THI). This is more pronounced and prolonged in premature infants because of decreased transplacental maternal IgG at birth. Infant with THI begins to experience recurrent respiratory tract infections and exhibit poor or absent antibody responses to vaccines. Spontaneous recovery occurs by 18 to 24 months. Some of these infants may benefit from intravenous immunoglobulin (IVIg) infusions or continuous antibiotic treatment. IVIg inhibits antibody formation and is contraindicated, if the infant is making antibodies.

Infections may be caused by pneumococci, *H. influenzae* or other pyogenic organisms. Chronic bacterial conjunctivitis may be an additional complaint. Chronic lung disease or intestinal malabsorption may be present.

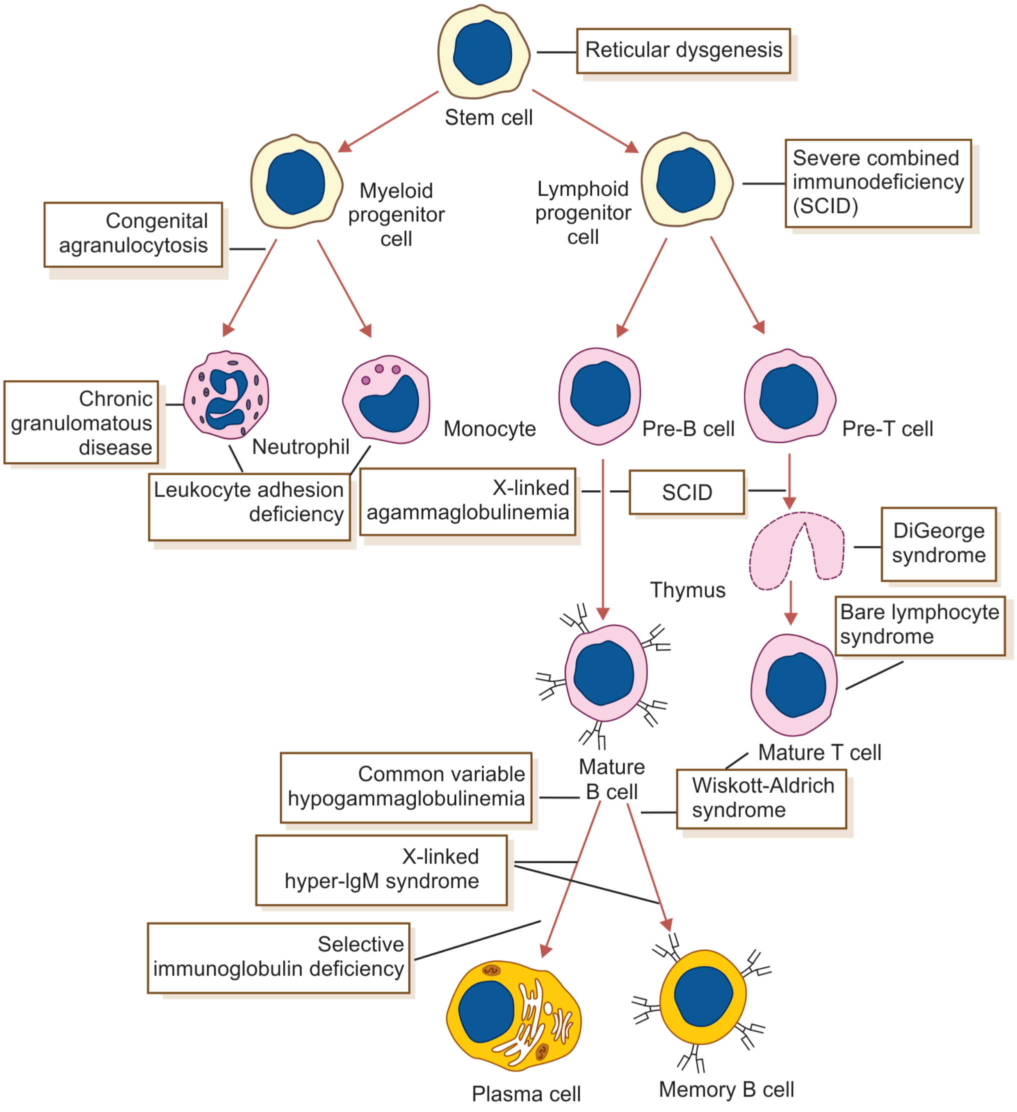

Reticular dysgenesis
Stem cell
Myeloid progenitor cell
Lymphoid progenitor cell
Severe combined immunodeficiency (SCID)
Congenital agranulocytosis
Chronic granulomatous disease
Neutrophil
Monocyte
Pre-B cell
Pre-T cell
Leukocyte adhesion deficiency
X-linked agammaglobulinemia
SCID
DiGeorge syndrome
Thymus
Bare lymphocyte syndrome
Mature T cell
Mature B cell
Common variable hypogammaglobulinemia
Wiskott-Aldrich syndrome
X-linked hyper-IgM syndrome
Selective immunoglobulin deficiency
Plasma cell
Memory B cell

**Fig. 13.2:** Reviews of overall cellular development in the immune system showing the location of defects that give rise to primary immunodeficiencies

only by 15 to 35 years of age. It is characterized by recurrent pyogenic infections. There is increased incidence of autoimmune disease. Malabsorption and giardiasis are common. Autosomal recessive mode of inheritance is observed in certain families. Total immunoglobulin level less than 300 mg/dL with the IgG level below 250 mg/dL. B cell numbers are usually normal, but they appear defective in being unable to differentiate into plasma cells and secrete immunoglobulins. The CD4/CD8 ratio may be reduced. Treatment is by administration of gamma globulins (IVIg, 400 mg/Kg).

### *Immunodeficiency with Hyper-IgM*

In immunodeficiency with hyper-IgM, levels of IgG and IgA are low, but there are elevated or normal level of IgM. Antibody function may be poor. Normal sequential development of immunoglobulins is initiated by IgM synthesis followed by IgG and IgA synthesis. The switching from IgM to IgG and IgA in B cells depends on binding of the B cell's CD40 to CD40 ligand on T cells. This deficiency is usually caused by mutation of the gene for CD40 ligand on T cells, which regulates switching from IgM to IgG and IgA in B cells.

The disease presents with recurrent pyogenic infections, including otitis media, pneumonia and septicemia. *Pneumocystis carinii* pneumonia is a frequent initial infection. Some patients have neutropenia, hemolytic anemia or aplastic anemia. Treatment is done with IVIg similar to that for XLA.

### *Selective Immunoglobulin Deficiencies*

*Selective IgA deficiency:* This deficiency could result from an arrest in the development of immunoglobulin-producing cells following the normal sequential development of IgM and IgG. The increased prevalence of recurrent sinopulmonary infections, gastrointestinal tract disease (ulcerative colitis, regional enteritis, celiac disease) and cancer may arise from the defective mucosal immunity to environmental microbial and other pathogens. Association of human leukocyte antigen (HLA) A1-B8 and DW3 has been found in patients with IgA deficiency.

Immunoglobulin A level in serum becomes below 15 mg/dL with other immunoglobulin levels remaining normal. IgA is absent in secretions.

Patients with selective IgA deficiency should not be treated with gamma globulins. Therapeutic gamma globulin contains only a small quantity of IgA and this is not likely to reach mucosal secretions through parenteral administration. Patients with recurrent infection should be treated aggressively with broad-spectrum antibiotics (Box 13.1).

**Box 13.1:** Evaluation of antibody-mediated immunity

- Protein electrophoresis
- Quantitation of immunoglobulins
- Enzyme-linked immunosorbent assay (ELISA)
- Specific antibody response
- B cell quantitation with monoclonal antibody

*Selective IgM deficiency:* It is a rare disorder associated with the absence of IgM and normal levels of other immunoglobulin classes. The cause of selective IgM deficiency is unknown. As a developmental disorder, absence of IgM with normal IgG and IgA contradicts the theory of sequential immunoglobulin development.

Patients with this disorder are susceptible to autoimmune disease and to overwhelming infection with polysaccharide containing organisms (e.g. pneumococci, *H. influenzae*) treatment should be with antibiotics.

*Selective deficiency of IgG subclasses:* Deletion of constant heavy chain genes or abnormalities of isotype switching may result in deficiencies of one or more of the IgG subclasses with normal or near of total IgG.

Patients have recurrent respiratory tract infections and repeated pyogenic sinopulmonary infections with *S. pneumoniae, H. influenzae* and *Staphylococcus aureus.* Sometimes this disorder is associated with other immunodeficiencies such as selective IgA deficiency or ataxia telangiectasia.

*Transcobalamin II deficiency:* Transcobalamin II, a vitamin B12 binding protein is necessary for the transport of vitamin B12 into cells. The patients, who lack this have hypogammaglobulinemia, macrocytic anemia, lymphopenia, severe intestinal malabsorption. Associated immunological defects are depleted plasma cells, diminished immunoglobulin levels and impaired phagocytosis. Specific antibody synthesis occurred following the administration of vitamin B12, but phagocytic activity is not restored.

*5′-nucleotidase deficiency:* This deficiency has been described in association with acquired hypogammaglobulinemia, X-linked hypogammaglobulinemia, Wiskott-Aldrich syndrome, AIDS and selective IgA deficiency. 5′-nucleotidase deficiency reflects diminished number of B cells or an abnormality in their maturation.

## T-cell Immune Deficiency Disorders

### *DiGeorge Anomaly (Congenital Thymic Aplasia, Immune Deficiency with Hypoparathyroidism)*

During 6 to 8 weeks of intrauterine life, the thymus and parathyroid glands develop from epithelial evaginations of the third and fourth pharyngeal pouches. DiGeorge anomaly is the result of interference with normal embryonic development at approximately 12 weeks of gestation. The most frequent presenting sign in patients with DiGeorge anomaly occurs in the first 24 hours of life with hypocalcemia that is resistant to standard therapy. Neonatal tetany and various types of congenital anomalies may also be present. Most patients develop recurrent and chronic infections with viral, bacterial, fungal or protozoal organisms. Failure to thrive may be present.

This primarily involves CMI. Thymus-dependent areas in spleen, lymph node are depleted of lymphocytes. Circulating T cells are reduced in numbers. Delayed hypersensitivity and graft rejection are depressed. Humoral immune mechanism is unaffected. Primary response is normal to many stimuli, but secondary response is impaired. Treatment is by transplantation of fetal thymus tissue (Box 13.2).

**Box 13.2:** Evaluation of cell-mediated immunity

- Enumeration of T cells (number and subtype) by flow cytometry
- Migration inhibitory factor testing
- Delayed type of hypersensitivity testing
- Mitogen stimulation by phytohemagglutinin and concanavalin A (PHAX con A)
- Detection of cytokine production from lymphocytes as index of functions

### *Chronic Mucocutaneous Candidiasis*

Chronic mucocutaneous candidiasis develop severe chronic candidiasis of mucosa, skin and nails. They often have endocrinopathies. CMI to *Candida* is deficient. Skin tests to *Candida* antigens will be negative despite chronic candidal infection. Intracellular killing of *Candida* is defective. This disorder is a selective defect in T cell immunity resulting in susceptibility to chronic candidal infection. Treatment is by transfer factor therapy along with amphotericin B.

### *Purine Nucleoside Phosphorylase Deficiency*

Purine $\xrightarrow{\text{PNP}}$ hypoxanthine $\longrightarrow$ uric acid. Patients, who have purine nucleoside phosphorylase (PNP) deficiency as an autosomal recessive, show decreased CMI followed by recurrent or chronic infections. They usually present with hypoplastic anemia, recurrent pneumonia, diarrhea and candidiasis. Low serum uric acid level point out the diagnosis.

## Combined Immune Deficiency

### *Cellular Immunodeficiency with Abnormal Ig Synthesis (Nezelof's Syndrome)*

In Nezelof's syndrome, there is decreased CMI associated with selectively elevated, decreased or normal levels of immunoglobulin. There is marked deficiency of T cell immunity and varying degrees of B cell immunodeficiency. Patients are susceptible to recurrent fungal, bacterial, viral and protozoal diseases. Abundant number of plasma cells are seen in spleen, lymph nodes, intestine and else where in the body. Thymic dysplasia with lymphoid depletion is seen. Autoimmune processes such as hemolytic anemia are common inspite of normal levels of immunoglobulins. Antigenic stimuli do not induce antibody formation. Treatment requires histocompatible bone marrow transplantation, thymus transplantation, transfer factor with adequate antimicrobial therapy.

### *Ataxia Telangiectasia*

Ataxia telangiectasia is hereditary, autosomal recessive in nature. It is associated with cerebellar ataxia, telangiectasia, ovarian dysgenesis and chromosomal abnormalities. Earliest signs are ataxia, choreoathetoid movements usually noticed in infancy. Telangiectasia involving conjunctiva, face and other parts of the body usually appears at 5 or 6 years of age. Death occurs due to sinopulmonary infection early in life or malignancy in 2nd or 3rd decade. Majority of them lack serum and secretory IgA and some possess antibody to IgA. IgE deficiency is also frequently seen. CMI is defective and there will be impairment of delayed hypersensitivity and graft rejection. Disease is progressive with neurological defects and immunodeficiency becoming more severe with time. Treatment is with transfer factor and transplantation of thymus.

### *Wiskott-Aldrich Syndrome*

Wiskott-Aldrich syndrome is X-linked inherited syndrome characterized by eczema, thrombocytopenic purpura and recurrent infections. Affected boys rarely survive in 1st decade of life. Death is due to infection, hemorrhage or lymphoreticular malignancy. CMI undergoes progressive deterioration associated with cellular depletion of the thymus and the paracortical areas of lymph nodes. Serum IgM level is low, but IgG and IgA levels are normal or elevated. Isohemagglutinins are absent in serum. Humoral defects result in specific inability to respond to polysaccharide antigens.

Treatment needs bone marrow transplantation and transfer factor therapy.

### *Immunodeficiency with Thymoma*

Immunodeficiency with thymoma usually occurs in adults, it is a benign thymic tumor. There is impaired CMI, with agammaglobulinemia. Aplastic anemia is frequent accompaniment.

*Episodic lymphopenia with lymphocyte toxins:* This is episodic, but profound depression of T cell function occurs by the action of circulating complement-dependent lymphocytotoxin. Toxin is antilymphocyte antibody. Patients lack immunological memory, so secondary antibody response is abolished. This disease has familial inheritance.

### *Severe Combined Immunodeficiency*

Severe combined immunodeficiency (SCID) is a group of disorders that arise from defects in lymphoid development that affect either T cells or both T and B cells. All forms of SCID have common features despite the underlying gene defects. Clinically, SCID is characterized by extreme lymphopenia and failure of T cells to mount immune response against microorganisms. The myeloid and erythroid cells appear normal in number and function.

There is a severe recurrent viral, fungal and protozoal infection, in the early years of life, because of defect in T cells. Initially, B cell defect is not evident in first few months, because of passively transferred antibodies via placenta or through mother's milk. In most cases, the illness begins within first 3 months of life with respiratory tract infection, pneumonia (often due to *P. carinii*), thrush rashes, fever and diarrhea. The immune system is so compromised that live attenuated vaccine [Sabin polio, bacille Calmette-Guérin (BCG)] can cause infection and disease. Various specific defects have been described under SCID are:

1. Mutations in the gene encoding the gamma chain ($\gamma$ C) of the interleukin-2 receptor (IL-2 Rr) lead to X-linked SCID. Defects in this chain inhibits signaling through receptors for IL-4, IL-7, IL-9, IL-15 as well as IL-2 receptors as the chain is present in receptors for all these cytokines.
2. Another form of SCID results from mutation of Janus kinase 3 (JAK3) and is inherited, as an autosomal recessive pattern. Deficiency of JAK3 will lead to unresponsive IL-2 receptors.
3. Mutation of $\alpha$ chain of IL-2 receptor may lead to a rare form of SCID, where T cell development will be affected.
4. Adenosine deaminase catalyzes adenosine to inosine. In its deficiency, there is accumulation of adenosine, which interfere purine metabolism.
5. Reticular dysgenesis is a severe form of SCID in which the defect lies in the multipotent stem cells. There is a total failure of myelopoiesis resulting in lymphopenia, neutropenia, thrombocytopenia and anemia.
6. About a third of SCID patients, there are defects in genes encoding recombinase activity proteins (RAG1 and RAG2), which are responsible for the rearrangement of deoxyribonucleic acid (DNA) that produced the variable regions of immunoglobulins and T cell receptors.
7. A variant of SCID, called 'bare lymphocyte syndrome', where there is general failure of immunity due to failure to transcribe the genes that encode MHC class II molecules. The inheritance is autosomal recessive. There is increased susceptibility to infection. There is defective intracellular signaling. CD4 T cell numbers are reduced. Immunoglobulin levels decreased owing to lack of T cell help.
8. Mutation of transporter associated with antigen presentation (TAP) genes cause deficiency of TAP-1 or TAP-2. There is decreased MHC class I expression and antigen presentation. In consequence, CD8 T cells number and function, decreased. There is increased susceptibility to viral infection and some intracellular bacterial infection.
9. Besides the conditions stated above, the other forms of SCID include:
    i. Surface receptor/transduction defect.
    ii. Tyrosine kinase ZAP-70 deficiency with non-functional CD4.
    iii. Defective cytokine synthesis (IL-1, IL-2).
    iv. Interferon-gamma (IFN-$\gamma$) receptor defect, etc.

## DISORDERS OF COMPLEMENT DEFICIENCY

### Complement Component Deficiency

Autosomal recessive mode of transmission is seen in this disorder. It is frequently associated with systemic lupus erythematosus (SLE). Complement component 3 (C3) deficiency is associated with recurrent pyogenic infections. C5, C8 deficiencies are associated with neisserial infections.

## Complement Inhibitor Deficiency

Complement 1 inhibitor deficiency leads to angioneurotic edema. It is transmitted as autosomal dominant. C3b inactivation deficiency is associated with chronic recurrent pyogenic lesions. Treatment is with androgens and aminocaproic acid (Box 13.3).

**Box 13.3:** Evaluation of complement deficiencies

- Hemolytic assay
- Immunochemical assay for complement components (C3, C4, C1 inhibitor and factor B)
- Nephelometry
- ELISA

## DISORDERS OF PHAGOCYTOSIS

Disorders of phagocytosis may be due to intrinsic or extrinsic defects. Intrinsic defects are the defects within phagocytic cell (e.g. enzyme deficiencies). Extrinsic defects are due to:

1. Deficiency of opsonic antibody, complement and other factors promoting phagocytosis.
2. Effect of drugs.
3. Antineutrophil autoantibodies.

Phagocytic dysfunction leads to increased susceptibility to infection ranging from mild recurrent skin infections to overwhelming systemic infections. Evaluation of phagocyte function is given in Box 13.4.

**Box 13.4:** Evaluation of phagocyte function

- Nitroblue tetrazolium dye reduction test
- Peripheral blood smear
- Bone marrow aspirate smear
- Phagocytic index
- Neutrophil adhesion assay
- Assay of chemotaxis
- Degranulation assay
- Bactericidal assays

### Chronic Granulomatous Disease

Chronic granulomatous disease is a familial disease manifests as recurrent infections with low-grade pathogens starting early in life. Progress of the disease is chronic and outcome is fatal. Chronic granulomatous lesions occur in the skin and lymph nodes along with hepatosplenomegaly. Catalase-positive pyogenic pathogens are the causative agents in the infections, because leukocytes from patients are unable to kill catalase-positive bacteria following phagocytosis. Diminished bactericidal capacity of some metabolic process such as oxygen consumption, hexose monophosphate pathway (HMP) activity and production of hydrogen peroxide ($H_20_2$), appear to be the major reasons for the bactericidal defects. Leukocytes do not undergo degranulation following phagocytosis. Delayed granule rupture and defective release of myeloperoxidase leads to inefficient bactericidal activity. Leukocytes from the patients fail to reduce nitroblue tetrazolium (NBT) dye during phagocytosis. This property has been used as a screening method for diagnosis of chronic granulomatous disease (NBT test). Two types of inheritance are seen. X-linked inheritance seen commonly in boys and rare. Autosomal recessive inheritance is commonly seen in girls.

### Myeloperoxidase Deficiency

Myeloperoxidase deficiency is a rare disease, here leukocytes have reduced myeloperoxidase. Patients are particularly liable to *Candida albicans* infection.

### Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome is a genetic disorder characterized by decreased pigmentation of skin, eyes and hair, photophobia, nystagmus and giant peroxidase-positive inclusions in the cytoplasm of leukocytes. Inclusions may be the result of antiphagocytic activity. Diminished phagocytosis activity is noticed in leukocytes. Patients suffer from frequent and severe pyogenic infections.

### Leukocyte G6PD Deficiency

Leukocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency is rare disease. Here leukocytes are deficient in G6PD. There is diminished phagocytic and bactericidal activity. NBT test is normal.

### Job's Syndrome

Job's syndrome is characterized by multiple large staphylococcal cold abscesses containing abundant quantity of pus, occurring repeatedly on skin and in various organs with little inflammatory response. Atopic eczema, chronic nasal discharge and otitis media are common features. Serum immunoglobulins are normal except for elevated IgE. It is probably a primary defect in phagocytic function.

### Tuftsin Deficiency

Tuftsin is chemically small tetrapeptide. Patients with tuftsin deficiency are prone to local and systemic bacterial infection.

### Lazy Leukocyte Syndrome

In lazy leukocyte syndrome, basic defect is in chemotaxis and neutrophil mobility. Bone marrow study reveals enough neutrophils, yet there is neutropenia with poor leukocyte response to chemical and inflammatory stimulation. Patients are more prone to infection with recurrent stomatitis, gingivitis and otitis.

#### *Hyper-IgE Syndrome*

The patients have an early onset of eczema and recurrent bacterial infection such as abscesses, pneumonia. Secondary infection of eczema is usually caused by *S. aureus* and *S. pyogenes*. Cellular and humoral immune responses are normal, but serum IgE level are usually increased 10 times than normal.

### Actin-binding Protein Deficiency

Frequent infection and slow mobility of leukocytes result from the defective action of binding protein.

### Shwachman's Disease

Frequent infections are found together with decreased neutrophil mobility, pancreatic malfunction and bone marrow abnormalities.

### Leukocyte Adhesion Deficiency

In leukocyte adhesion deficiency condition, there is deficiency of cell surface molecules belonging to the integrin family of proteins, which functions as adhesion molecules and are required to facilitate cellular interaction. Three of these (LFA-1, Mac-1 and gp150/95) have a common β-chain (CD18) and are variably present in different monocytic cells. The immunodysfunction is related to defect in β-chain affecting expression of these three molecules.

## SECONDARY (ACQUIRED) IMMUNODEFICIENCIES

Certain immunodeficiency diseases, instead of arising from genetic or developmental causes, may result from environmental exposure. These diseases are termed as secondary immune deficiencies. Among the environmental factors that affect adversely on the immune system are general health, therapeutic treatment, infections and malignancies.

### GENERAL HEALTH (PHYSIOLOGICAL SEQUELAE)

Several factors may adversely affect the immune system. Stress leads to depressed immune functions. Among the most studied factor is the nutrition. Malnutrition affects both antibody-mediated immunity (AMI) and CMI in eliminating the organisms. Reduced level of vitamins (A, B6, C and E) and trace elements (iron, zinc and selenium) are associated with impaired immune functions. Amino acid, glutamine is critical for energy metabolism. Postvaccination immune responses are higher in subjects given nutritional supplements than in untreated, control and malnourished. Probiotics benefit health and immunity. Old age leads to secondary immune deficiency because of immunosenescence.

## THERAPEUTIC TREATMENT

A normal individual immune system may be suppressed as a side effect of medical treatment. A transplant recipient is more prone to suffer from opportunistic infections. Corticosteroids, in the treatment of autoimmune diseases, interfere the immune response by depletion of lymphocytes and there by reduction of cytokines. Cytotoxic drugs or radiation treatment for various cancers, frequently damage the dividing cells of the body including those of the immune system.

## MALIGNANCIES

In lymphoma and leukemia, the normal functional lymphocytes are overgrown by cancerous lymphocytes, thus reducing the immune system's ability to respond to different antigens. The malignant cells often, begin to display aberrant surface molecules and alter their normal production of cytokines, antibodies and other molecules.

## INFECTION

Many infectious agents evade or circumvent the immune response generated against them. In many cases, the manner in which the evasion takes place leaves the host, more prone to other infectious agents. For example, some bacteria secrete enzymes, which destroy the local immunoglobulin and complement components. Some bacteria and viruses protect themselves after ingestion by phagocytes by inhibition of several key phagocytic activities.

Specific immune suppression may occur as that happens in leishmaniasis. In this case, there is immune suppression of T cell ($T_h1$ cell) reactivity. Parasites can cause disruption of lymphoid cells or tissue directly (the soluble lymphotoxic factor of *Trichinella spiralis*). *Schistosoma* can cleave a peptide from IgG. Soluble parasite antigens can be liberated in enormous quantities by a process known as immune distraction. Non-specific immune suppression is a universal phenomenon of parasitic infection as a whole.

A large number of viruses evade host's immune mechanism by causing generalized immune suppression. Among them mumps, measles, Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and HIV are common. In some cases, the virus directly destroys the lymphocytes and macrophages (HIV causing lysis of CD4 cells). In other cases, immune suppression may be due to cytokine imbalance. For example, EBV produces a protein (BCRF-1), which has got similar action as IL-10, which suppresses $T_h1$ activity leading to decreased level of IFN-$\gamma$ and IL-2.

### HIV Infection and AIDS

Globally, as of December 2005, the total estimated number of people living with HIV was 40.3 millions. Most of HIV infected (25.8 millions) are living in Sub-Saharan area. India accounts for almost 10 percent of the 40 million people living with HIV/AIDS globally. Over 27 million people, worldwide, have died of AIDS, since the first case was identified in 1980. However, the new cases of AIDS and HIV related deaths have considerably decreased from early 1990 owing to the effective antiretroviral therapy (ART) in USA and Europe. The availability of antiviral therapy at other parts has improved and there is hope that it will produce similar effect.

The disease that HIV-1 causes, AIDS was first reported in the United States in 1981 in Los Angeles, New York and San Francisco. A group of patients displayed unusual infections including by a pathogen (*P. carinii*) causing pneumonia. Some other groups, having AIDS had also a skin tumor called Kaposi's sarcoma. On complete evaluation it was found that these patients were deficient of cell-mediated immune response. On further study, it was found that T cells that carry CD4 receptors were sufficiently reduced in number. As the number of AIDS cases increased and the disease was recognized, throughout the world, it became evident that the high risk for AIDS were homosexual males, promiscuous heterosexual partners, intravenous drug users, persons who have received blood and blood products and the infants born to HIV infected mother.

### *Causative Agent of AIDS*

Following the recognition of AIDS as an infectious disease, the causative agent, a virus was discovered simultaneously by two stalwarts, Luc Montagnier in Paris and Robert Gallo in Bethesda. The agent was a retrovirus, which carries the genetic information in the form of ribonucleic acid (RNA). Following the entry of the virus into the cell, the RNA transcribed to DNA by a virally coded enzyme (reverse transcriptase). This DNA copy is called provirus, which is integrated into the cell genome and is replicated along with cell DNA. The provirus is expressed to form new virions, when the cell undergoes lysis. Alternately, the provirus remains in latent form until some regulatory signals initiates the expression process. Before the discovery of HIV, only one retrovirus that is human T cell lymphotropic virus-1 (HTLV-1) was known to cause T cell leukemia and HTLV-1 associated myopathy. Besides HIV-1, there is HIV-2, which is less pathogenic in humans. HIV-2 is similar to the virus isolated from monkey [simian immunodeficiency (SIV)].

### *Structure of Virus*

Human immunodeficiency virus is 120 n icosahedral, enveloped RNA virus. HIV comprises of an outer envelope consisting of lipid bilayer with uniformly arranged 72 spikes or knobs of gp120 and gp41. Glycoproteins 120 (gp120) protrude out and gp41 is embedded in the lipid matrix. Inside, two copies of RNA are surrounded by protein core. Protein core contains viral enzymes (reverse transcriptase, integrase and protease), which are essential for viral replication and maturation. Gp120 serves as the viral receptor for CD4 on host cells. The viral envelope derives from the host cells and contains some host cell membrane proteins including class I and class II MHC molecules. Within the envelope, is the viral core or nucleocapsid, which contain proteins called p17 and p24. Inside, the HIV genome consist of two copies of single-stranded RNA, which are associated with two molecules of reverse transcriptase (p64) and a nucleoid protein, p10 a protease and p32, an integrase (Fig. 13.3).

*Genetic structure:* The genetic structure of virus contains both highly conserved and variable regions. The high variability of the virus accounts for drug resistance, evasion of the immune response and impediments for preparation of vaccines. In an infected individual quasispecies of a particular viral subtype may be found on account of constant variability. HIV has two sets of genes, which code for structural and regulatory products. Structural genes direct the synthesis of the physical components of virus and are also responsible for viral size, shape and structural integrity. The regulatory genes direct synthesis of proteins that effect the synthesis of viral components. Structural genes are *gag, pol* and *env*. The regulatory genes along with some accessory genes are shown in Figure 13.4.

### *Routes of HIV Transmission*

Human immunodeficiency virus-1 spreads by several contact, infected blood and blood products, infected organs and from mother to

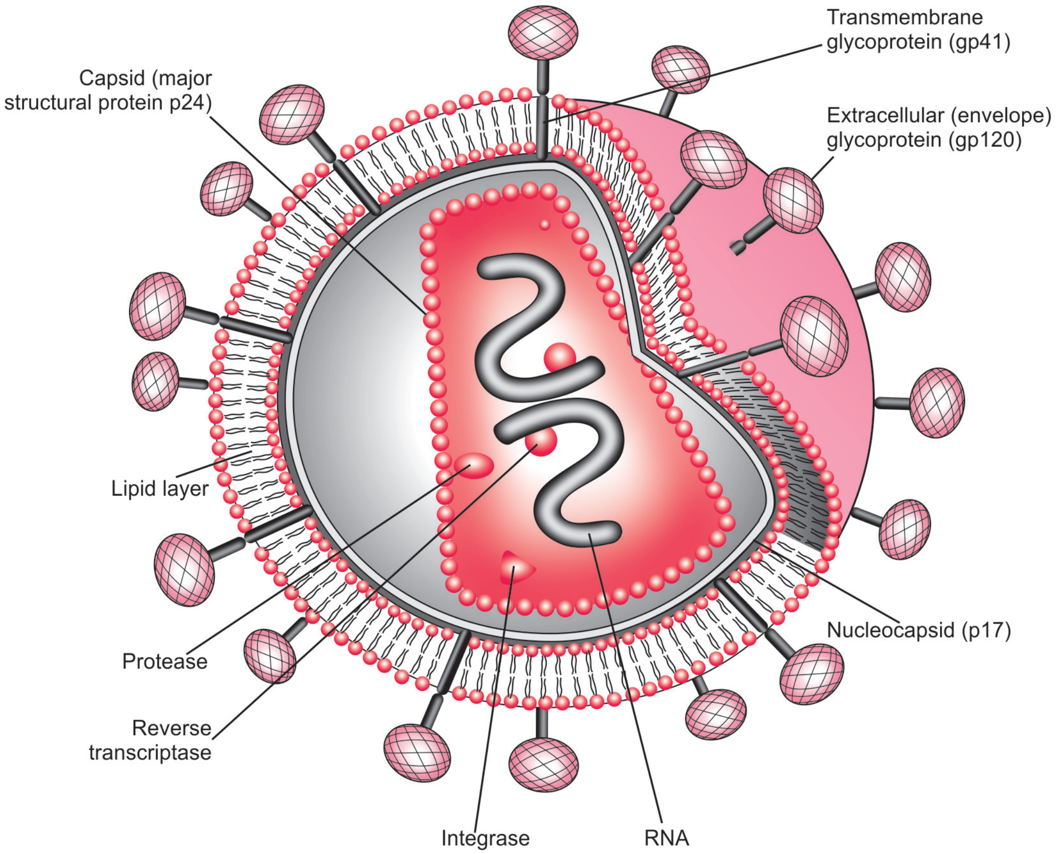

Transmembrane glycoprotein (gp41)
Capsid (major structural protein p24)
Extracellular (envelope) glycoprotein (gp120)
Lipid layer
Nucleocapsid (p17)
Protease
Reverse transcriptase
Integrase
RNA

**Fig. 13.3:** Human immunodeficiency virus (HIV)

fetus and infant. Virtually every well documented cases of HIV infection, there is evidence of contact with blood, milk, semen or vaginal fluid from an infected individual. The most efficient vehicle of HIV transmission is blood. However, the risk of infection via blood transfusion is now extremely low, due to strict HIV screening of donated blood. The most common route of transmission is unprotected penetrative sexual contact. Different forms of sexual practices carry a variable risk gradient of acquiring HIV. Cells associated rather than the free virus is responsible for disease transmission. Anal intercourse carries a high-risk of transmission. Risk to insertive partner is through the infection of lymphocytes and macrophages in the foreskin or along the urethral canal. In females, HIV transmission occurs when infected cells in semen gain entry into the female genital tract. The likely hood of infection is greatly enhanced by the presence of sexually transmitted disease (STD). Lesions and open sores present in many STDs favor the transfer of HIV infected cells to either partner. Possible vehicles of passage from mother to infant include blood transferred in birth process and milk in the nursing period. Exposure to in-

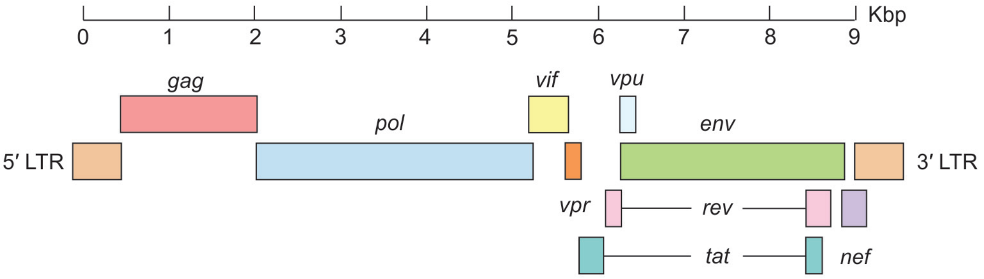


**Fig. 13.4:** Genetic organization of HIV-1. The three major genes: *gag, pol, env* encode polyprotein precursors that are cleaved to yield the nucleocapsid core protein, enzymes required for replication and envelop core proteins. The non-structural and regulatory genes include *tat* (transactivating gene), *nef* (negative factor gene), *rev* (regulator of virus gene), *vif* (viral infectivity gene), *vpu* (enhance maturation gene), *vpr* virus proneotor gene). LTR, long terminal repeat.

fected blood accounts for the high incidence of AIDS in intravenous drug users, who normally share hypodermic needle. Figure 13.5 depicts the different routes of transmission of HIV.

### Cell Attachment and Replication

The first step in the HIV infection is the binding of the virus (gp120) to the host cell receptor, CD4 molecule, present in the T cells and macrophages. This interaction is not sufficient for entry and productive infection. Expression of other cell surface molecules (CXCR4 for T cells and CCR5 for macrophages) are essential for productive infection (Fig. 13.6).

Following the entry of the virus, the RNA genome of the virus is transcribed to a cDNA (provirus) by an enzyme called reverse transcriptase. The integrated provirus is transcribed and various viral messages are translated into proteins, which along with a complete new copy of the RNA genome are used to form new viral particles. The *gag* proteins of the virus are cleaved by the viral protease into the forms that make up nuclear capsid in a mature infectious viral particle.

A T-tropic HIV-1 strain uses CXCR4, while the M-tropic HIV-1 strain uses CCR5. The use of different coreceptors, by HIV-1, also explains the different role of cytokines on viral replication. While some chemokines have a negative effect, on viral replication some such as proinflammatory cytokines had positive effect. Both the HIV coreceptors (CXCR4 and CCR5) are also receptors for chemokines. Some chemokines compete with HIV-1 and block the binding of HIV to the T cell, thus preventing the entry. On the other hand, some proinflammatory cytokines express more chemokines on the cell surface, making the cells more susceptible to viral entry.

### Viral Pathogenesis and Immune Response

Human immunodeficiency virus and HIV-infected cells reach the lymph nodes and other lymphoid tissues, which are the sites of active immune response against viral antigens. T lymphocytes are activated on account of infection, but HIV replicates better in the activated cells. The peak in number of virus-expressing cells and the spread of virus throughout the lymphoid tissue precedes the increase in plasma viremia that is the virus in the blood. The virus spills over from lymph node. In the first 2 to 3 weeks after infection,

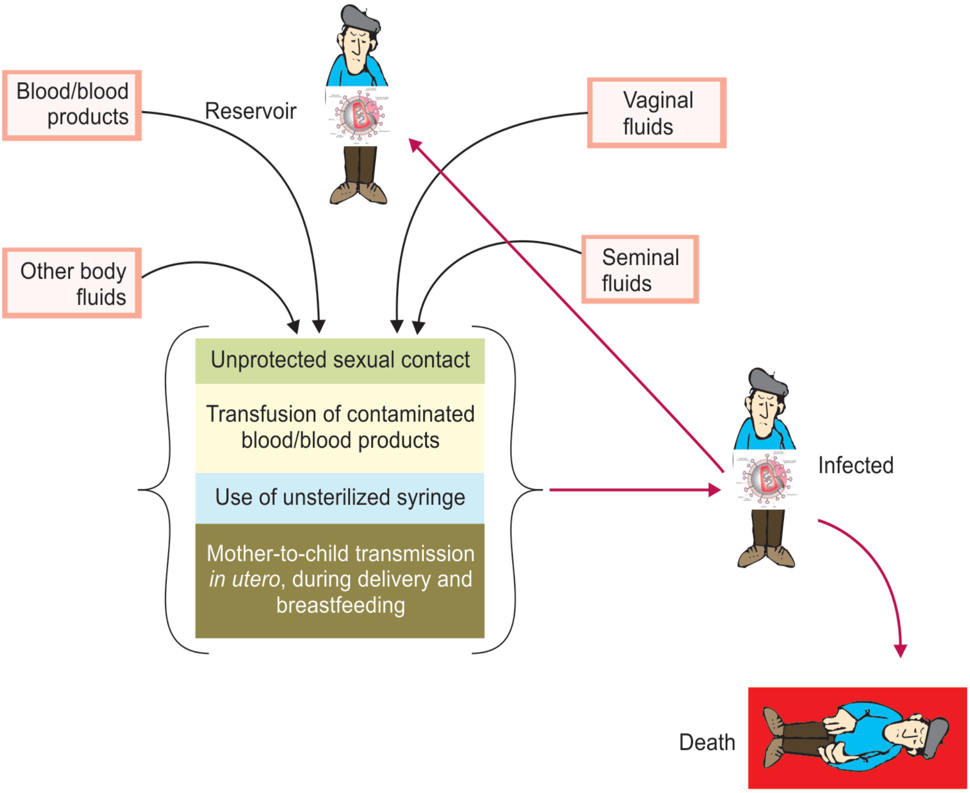

Blood/blood products
Reservoir
Vaginal fluids
Other body fluids
Seminal fluids
Unprotected sexual contact
Transfusion of contaminated blood/blood products
Use of unsterilized syringe
Mother-to-child transmission *in utero*, during delivery and breastfeeding
Infected
Death

**Fig. 13.5:** Transmission cycle of HIV

there is intense virus spreading and therefore this period is called stage of virus dissemination, which coincides clinically with 'flu-like illness', lymphadenopathy (acute HIV disease). In this stage, there is significant reduction of CD4 T cells and the viral level in the blood may be as high as $10^6$ to $10^7$/mL (stage of viremia).

The next stage is that of a down regulation of viremia because of robust immune response, carried out by HIV-specific cytotoxic T lymphocytes (CTL) and humoral response carried out by complement fixing and neutralizing HIV-specific antibodies. The period from entry of HIV to the appearance of detectable amount of antibodies is known as window period. In this period, the individual is infected, infectious to others, but seronegative. The window period ranges from 3 weeks to 3 months. Both HIV-specific antibodies and CTL, kill the virus infected cells. The viremia drops and the number of CD4 cells bounce back to a level slightly lower than the previous level (Fig. 13.7).

Although immune response succeeds down regulating the viremia, HIV is never completely eliminated. Thus, the stage passess on to this chronic stage. The factors, which determine the progression of HIV infection are:

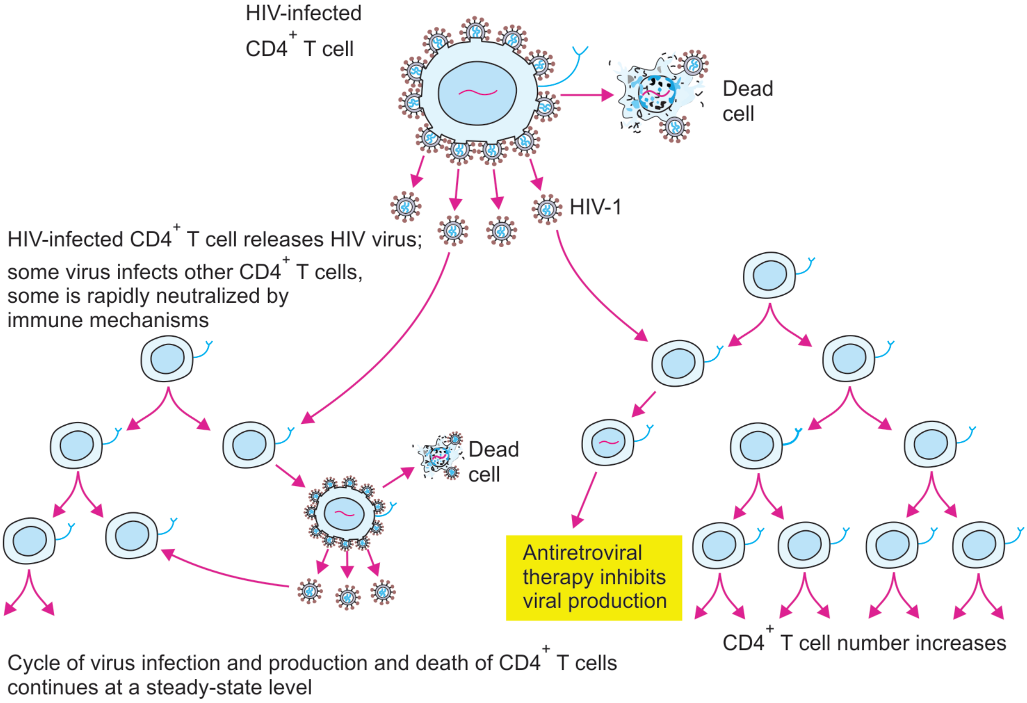

HIV-infected CD4⁺ T cell
Dead cell
HIV-1
HIV-infected CD4⁺ T cell releases HIV virus; some virus infects other CD4⁺ T cells, some is rapidly neutralized by immune mechanisms
Dead cell
Antiretroviral therapy inhibits viral production
CD4⁺ T cell number increases
Cycle of virus infection and production and death of CD4⁺ T cells continues at a steady-state level

**Fig. 13.6:** Cell attachment and replication of HIV

1. Quality of T cell response (genetically determined).
2. Number of permissive cells.
3. Previous infection by CMV, EBV and herpes virus infections.

### *Asymptomatic Stage (Clinically Latent Period)*

Asymptomatic stage is marked by down regulation of viremia and disappearance of symptoms of acute viral disease. All the virological parameters in the peripheral blood (viral RNA copies/viral load, virus expressing mononuclear cells, etc.) are very low. However, active and continuous viral replication goes in the lymph nodes and other lymphoid organs. As long as CD4 cell count is 500/mL, the immune response is effective. The important paradox is that though the activation of T lymphocyte is aimed at the elimination of virus, the replication of virus is augmented in activated CD4 cells. So there is gradual reduction in the number of CD4 cells and increase in virus load during the long stage. Increase in virus load in peripheral blood indicates failure and progressive deterioration of effective immune response.

Humoral immunity remains unaffected during this asymptomatic period, which lasts for 8 to 10 years. Though antibodies are produced against various proteins of HIV, they are unable to clear the virus because of constant variation and cell-to-cell transmission. Progressive impairment of HIV specific and non-specific cell-mediated and humoral responses force the onset of AIDS. The CD4 count become less and less (200–500) and

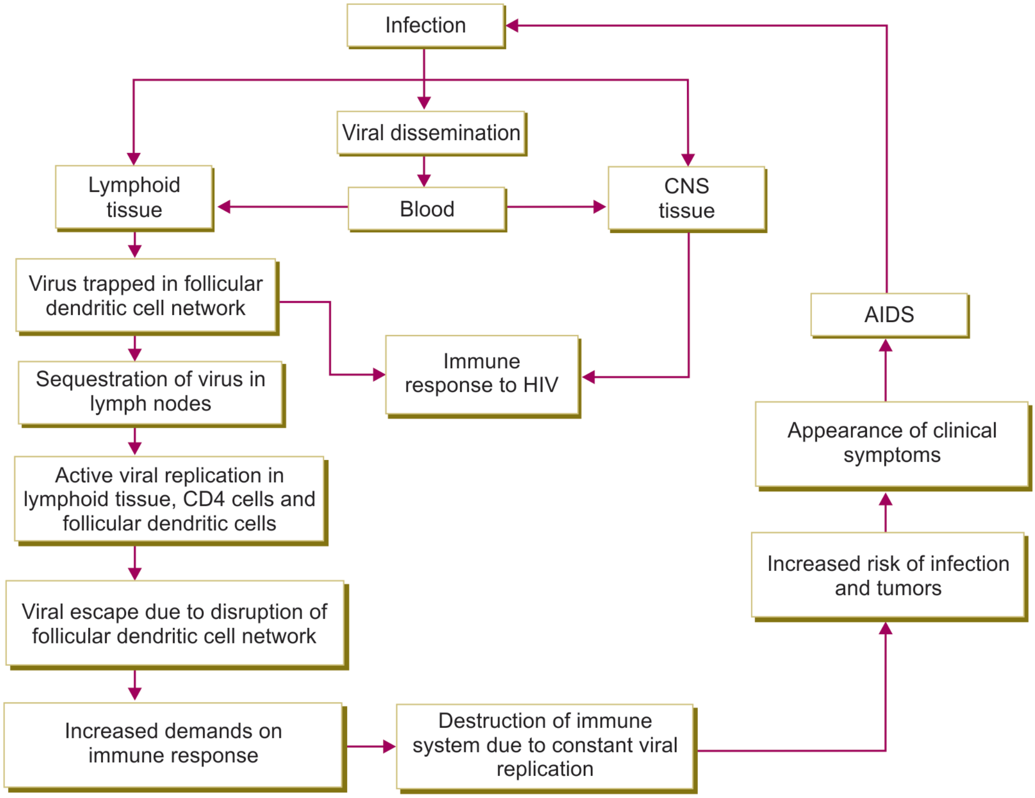

Infection
Viral dissemination
Lymphoid tissue
Blood
CNS tissue
Virus trapped in follicular dendritic cell network
Sequestration of virus in lymph nodes
Active viral replication in lymphoid tissue, CD4 cells and follicular dendritic cells
Viral escape due to disruption of follicular dendritic cell network
Increased demands on immune response
Destruction of immune system due to constant viral replication
Immune response to HIV
AIDS
Appearance of clinical symptoms
Increased risk of infection and tumors

**Fig. 13.7:** Viral pathogenesis and immune response

the viral load/RNA copy becomes more and more (Fig. 13.8).

### *Acquired Immunodeficiency Syndrome*

The course of HIV infection begins with no detectable anti-HIV antibodies or virus-specific antibodies and progress to the full AIDS syndrome. The diagnosis of AIDS include evidence for infection with HIV (presence of antibodies or virus in blood), greatly diminished number of CD4 cells (< 200 cells/mm$^3$), impaired or absent delayed hypersensitivity reactions and the occurrence of opportunistic infections. Patients of AIDS usually succumbs to tuberculosis, pneumonia and severe wasting diarrhea or/and malignancies.

### *Mechanism of CD4 T Cell Depletion and Dysfunction*

CD4 T cells are the main targets of HIV and progressive destruction of these cells is the characteristics of all stages of HIV disease. CD4 cells are surrogate markers to monitor the progression of HIV infection. These cells are destroyed by certain mechanisms.

*Direct damage by virus:* HIV can kill singly or after giant cell and syncytia formation. Increase in cell permeability to the budding of newly formed virus, which punches holes and kills the virus. Single cell killing occurs due to accumulation of integrated viral DNA and inhibition of cellular protein synthesis.

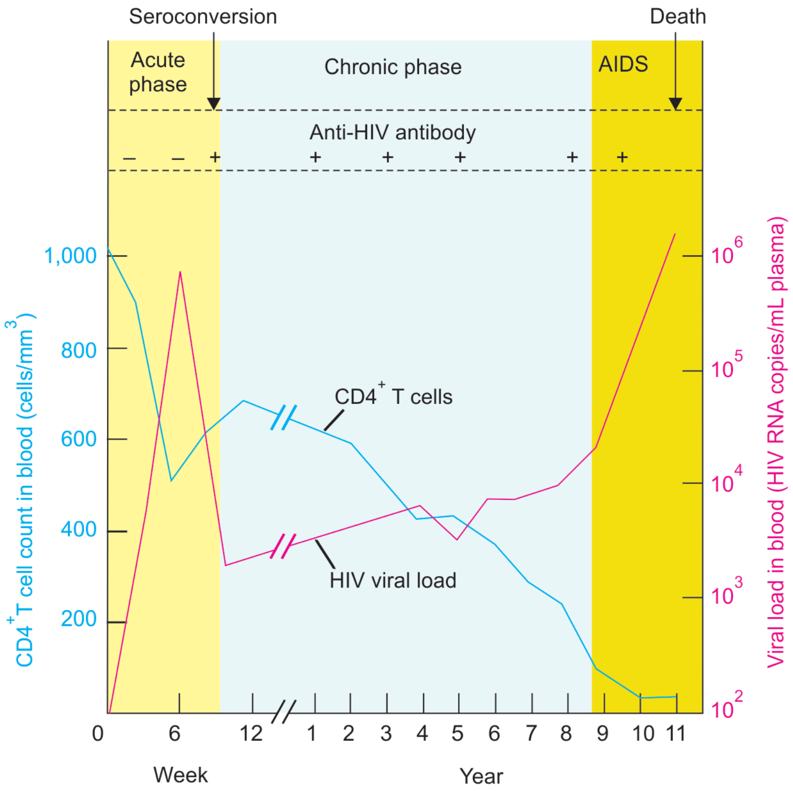


**Fig. 13.8:** Serologic profile of HIV infection showing three stage in the infection process. Soon after infection, viral RNA is detectable in the serum. However, HIV infection is most commonly detected by the presence of anti-HIV antibodies after seroconversion, which normally occurs within a few months after infection. Clinical symptoms indicative of AIDS generally do not appear for at least 8 years after infection, but this interval is variable. The onset of clinical AIDS is usually signaled by decrease in T cell numbers and an increase in viral load (*Adapted from A* Fauci, et al. 1996, Annals Int. Med. 124:654).

CD4 cells expressing viral antigens on the surface attract CD4 uninfected cells and the membranes of these fuse to form giant cells and syncytia. Thus, one such HIV infected cells can eliminate hundreds of uninfected cells by syncytia formation.

*Immune mechanisms triggered during the course of HIV infection:* The non-virologic mechanisms, which can damage/destroy CD4 cells include autoimmune phenomenon involving CD4 molecules, antibody-dependent cellular cytotoxicity (ADCC), anergy, superantigens and death of innocent bystander cells.

### *Therapeutic Agents*

There are several strategies for development of effective antiviral drugs. The key to success of such therapy is that they must be HIV specific without or minimal interference in the normal cellular process.

The first successful attempt was with the drugs that will interfere with the reverse transcriptase of RNA to cDNA. Prototype of drugs, which interferes with reverse transcriptase is zidovudine (azidothymidine, a nucleoside analog, which causes termination of chain).

The second stage of viral replication that has proved amenable to blockade is the step at which the precursor proteins are cleaved into the units needed for construction of a new mature virions. This step requires the action of specific viral protease, which can be inhibited by chemical agents.

Current treatment for AIDS is a combination therapy, designated as high active antiretroviral therapy (HAART), which includes two nucleoside analogs and one protease inhibitor. The use of immune modulator, IL-2, in conjunction with HAART is being examined, as a strategy to restore normal immune response. Research is going on for drugs in various stages of development. While one class of drugs interferes with the integration of viral DNA into the host genome, the others being considered, which prevents the attachment of virus to the host cell.

### *HIV/AIDS Vaccines, Trials and Constraints*

The AIDS epidemic continues to spread, despite the advances in therapeutic approach. Present use of HAART, because of its high expense and its side effects, cannot have a universal application. This may be helpful for an individual for regression and recovery, but it cannot eradicate the AIDS epidemic particularly in developing countries. Therefore, the most sorted option is the vaccine for the AIDS. But then, what are the constraints or the impediments for which a suitable vaccine for providing long-term immunity, has not yet been possible though the causative agent has been isolated, since 1980.

*Absence of natural immunity in AIDS:* Most effective vaccines mimic the natural state of immunity. Persons, who recover from the infection develop some immunity to the particular infection. In HIV-1 infection, though antibodies are produced against the proteins of the virus, progression to immunodeficiency states continues. Immunity may restrain the virus, but rarely exceeds for more years. In rare subset of infected individuals, called long-term non-progressor, the period of infection without disease is longer may be indefinite.

*HIV integrates in host genome and remains in latent form:* Polio and influenza vaccine may hold the virus produced by the infected cells as an inative form, so that it does not cause harm to the host. The vaccine prevents entry and multiplication at the site of infection. HIV-1 is integrated to the host genome and remains latent for years. Complete clearance of virus is almost impossible by humoral and cell-mediated immunity.

*Instability of HIV genome:* Barring the influenza and rhinoviruses, most other viruses exhibit minor variability in structure. It is easier to findout candidate antigen for preparation of vaccine, which will be effective in generating appropriate immune response. HIV-1, on the other hand, shows variation in most viral antigens and the rate of replication may be, as high as $10^9$ viruses per day. This variability along with rapid replication will produce many mutant viruses (quasispecies), which escape immunity.

*Difficulty in preparation of live attenuated vaccines:* Except recombinant hepatitis B and conjugate used for *H. influenzae* type b, majority of the viral vaccines are live attenuated or heat-killed organisms. The problem with the members of the retrovirus is that, the cDNA is integrated to the host genome. On the positive side, chemical study using other viruses such as attenuated vaccinia or canarypox, as carriers, for genes encoding HIV proteins have passed phase I (safety) trials and have advanced to phase II (efficacy) trials.

*HIV is encountered frequently and potentially, in large doses:* For many viruses, the frequency of exposure is rare or seasonal. Therefore, vaccination has been successful. But incase of high-risk individuals, such as sex workers, intravenous drug users, the exposure to HIV is frequent for longer time and also in large doses. Thus, HIV vaccine is required to prevent infection against a constant attack of viruses in large doses. This is most unlikely that the HIV vaccine would serve the purpose.

*Doubtful mucosal protection for HIV:* Many vaccines have been successfully, designed to protect gastrointestinal (Sabin polio) and respiratory tract (influenza vaccine) infections. Most common route of HIV infection is by the genital tract. It is not known, whether the mucosal immunity will protect against infections by this route. Preliminary vaccine studies, using rectal or vaginal challenge of immunized primates with HIV, simian immunodeficiency virus (SIV), chimeric viruses simian-human immunodeficiency virus (SHIV) show protection to this challenge route.

*Animal studies denied hope for HIV vaccines:* Development of most vaccines relies upon animal studies, then clinical trials. Animal studies of HIV infection and disease have yielded disappointing result. The immune responses are neither protective nor prevent progression of the disease. Many results involve a specific virus in a particular host and are not easily extrapolated to universal concept. However, in one study it was seen that the passive immunization of antibody from HIV-infected chimpanzees protect macaques from challenge with SHIV strains bearing HIV-envelope glycoprotein.

*Vaccine trials:* No reports of great success is seen in human HIV-vaccine trials. Since the last report, 60 phase I trials were undertaken, involving recombinant proteins, peptides, DNA vaccine and poxvirus/recombinant protein combinations. At the same time, six phase II trials were in progress and only two candidates advanced to phase III or final phase of clinical trials—the test of efficacy. Despite sincere efforts from all over the world, hope of inventing a suitable vaccine remains elusive.

Development of vaccine for HIV and AIDS is a challenge and a necessity. Some works have been done. More research is needed to understand, how this viral attack on the immune system can be thwarted. An intense and cooperative effort must be directed to devise, test and deliver a safe and effective vaccine for AIDS.

As no cure or vaccine is currently available, our main weapon is prevention through health education and control of infection.

## STUDY QUESTIONS

### Essay Questions

1. Summarize the causes of immune deficiencies. What is the effect of an immune deficiency?
2. Classify immunodeficiency. How to evaluate various immunodeficiency states?
3. Describe the pathogenesis and immune response of HIV infection (AIDS).
4. Mechanism of CD4 T cell depletion and dysfunction in HIV infection.

### Short Notes

1. Bruton's agammaglobulinemia.
2. DiGeorge anomaly.
3. Reticular dysgenesis.
4. Chronic granulomatous disease.
5. Chédiak-Higashi syndrome.
6. Job's syndrome.
7. Window period in HIV infection.
8. Methods of HIV transmission.

## SUGGESTED READING

1. A genome–wide search for asthma susceptibility loci in ethnically diverse populations. The collaborative study on the genetics of asthma (SGA). Nat Genet. 1997;154: 389-92.

2. Biron CA. Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. Semin Immunol. 1998; 105:383-90.
3. Black JG. Microbiology Principles and Applications, 3rd edition. USA: Printice Hall College Div; 1996.
4. Borish L. Genetics of allergy and asthma. Ann Allergy Asthma Immunol. 1999;825: 413-24.
5. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
6. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
7. Greenwood D, Slack R. Medical Microbiology, 15th edition; 1997.
8. Jawetz. Textbook of Microbiology, 25th edition; 2010.
9. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby, Elsevier; 2006.
10. Stites. Basic and Clinical Immunology, 8th edition. 2007.
11. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
12. Tortora, Funke, Case. Microbiology: an introduction, 6th edition. 1998.

# Tumor Immunology

# 14

In developing countries, the death toll from infectious diseases is on the decline. Amongst the other causes of death, death due to cancer ranked higher next only to heart disease. From an immunologic perspective, cancer cells can be viewed as altered self-cells that have escaped normal growth regulating mechanisms. This chapter deals with the unique properties of cancer cells that can be recognized by the immune system and the various mechanisms, how the immune system applies to get rid of the cancer cells as well as the methods how cancers manage to evade the mechanism. Finally, this chapter describes current clinical and experimental immunotherapies for cancer.

## CANCER: ORIGIN

In most matured organs and tissues, the renewal and death of the cells go simultaneously. The mature cells in the body have a lifespan. As these cells die, new cells are generated by proliferation and differentiation of stem cells. In normal circumstances, the generation of cells is so regulated that number of any particular cell types remain constant. In some pathologic conditions, stimulus for cell proliferation exceeds the requirement for cell replacement, resulting, organ hypertrophy from polyclonal expansion of cells proliferating in response to growth signals. Once the condition responsible for excessive cell growth terminates, however, the rate for cell proliferation decreases and the organ hypertrophy resolves. In non-malignant tumors there is regulated polyclonal growth. On the other hand, in malignant tumors, an individual cell may undergo a transforming event and acquire the potential to produce daughter cells that proliferate independent of external growth and regulatory signals. The autonomous growth of such transformed cells of monoclonal origin represents the basis of malignant disease. Amongst various malignant tumors, such as carcinoma, sarcomas, leukemia and lymphomas, the protean effects of cancer reflect in large part of the unrestrained growth of tumor cells that locally invade and disrupt normal tissues, as well as metastases and grow in distant organs.

### Malignant Transformation of Cells

When normal cultured cells are treated with chemical carcinogens, irradiation and certain viruses, there is alteration of morphology and growth and such process is known as transformation. These ‘transformed’ cells are capable of producing tumor, when they are injected into animals. Such, cells have undergone malignant transformation and they often exhibit properties similar to cancer cells. For example, they have decreased requirements for growth factor and serum, are no longer anchorage dependent and grow in a density independent fashion. Both cancer cells and transformed cells can be subcultured indefinitely. For all practical purpose, they are immortal.

Transformation may be induced by mutation in response to various chemical agents [deoxyribonucleic acid (DNA) alkylating agents] and physical agents (ultraviolet light and ionizing radiation). A number of DNA and ribonucleic acid (RNA) viruses have been shown to induce malignant transformation. The two best examples of DNA viruses, which induce malignant transformation are simian virus 40 (SV40) and polyomavirus. In both cases viral genome is integrated in host chromosomal DNA. The proteins encoded by these viruses (SV40: large T and little T; polyoma: large T, middle T and little T) play important role in the malignant transformation of cells. Most RNA viruses except retroviruses replicate in the cytoplasm and do not induce malignant transformation. The retroviruses transcribe their RNA into DNA by means of a reverse transcriptase enzyme and then integrate the transcripts into the host DNA. In some cases, retroviruses induce transformation of cells by oncogenes or cancer genes. One of the best studied transforming retrovirus is the Rous sarcoma virus, which brings about transformation by oncogenes.

### *Oncogenes and Cancer Inductions*

Oncogenes are not only present in the virus, which induce transformation, but are also found in normal cells. The oncogenes present in the cells are known as proto-oncogenes or cellular oncogenes (C-onc) and the oncogenes present in virus are called viral oncogenes (V-onc). It has become apparent that most if not all, oncogenes (both viral and cellular) are derived from cellular genes that encode various growth-controlling proteins. The proteins encoded by a particular oncogene and its corresponding proto-oncogene appear to have very similar functions.

## Function of Cancer-associated Genes

In normal tissue, cellular proliferation and cell death are guided by highly regulated process. If there is an imbalance either in cellular proliferation or in cell death, cancerous state will develop. Oncogenes and antioncogenes (tumor suppressor genes) play important role in regulating the proliferation or cell death. Three categories of cancer-associated genes are known (Figs 14.1A and B).

One category of proto-oncogene and their oncogenic counter part encode proteins, which induce cell proliferation. Some of these proteins function as growth factors or growth factor receptors. These genes include *sis,* which encodes a form of platelet-derived growth factor and others such as *fms, erbB* and *neu,* which encode growth factor receptors. In normal cells, the expression of growth factors and their receptors are carefully regulated. Inappropriate expression of growth factors or their receptors can result in uncontrolled proliferation. The *src* and *abl* oncogenes encode tyrosine kinases and the *ras* oncogene encodes a guanosine triphosphate (GTP) binding protein. The products of the gene act as signal transducers. The *myc, jun* and *fos* oncogenes encode transcription factors. Overactivity of any of these genes may result in unregulated proliferation.

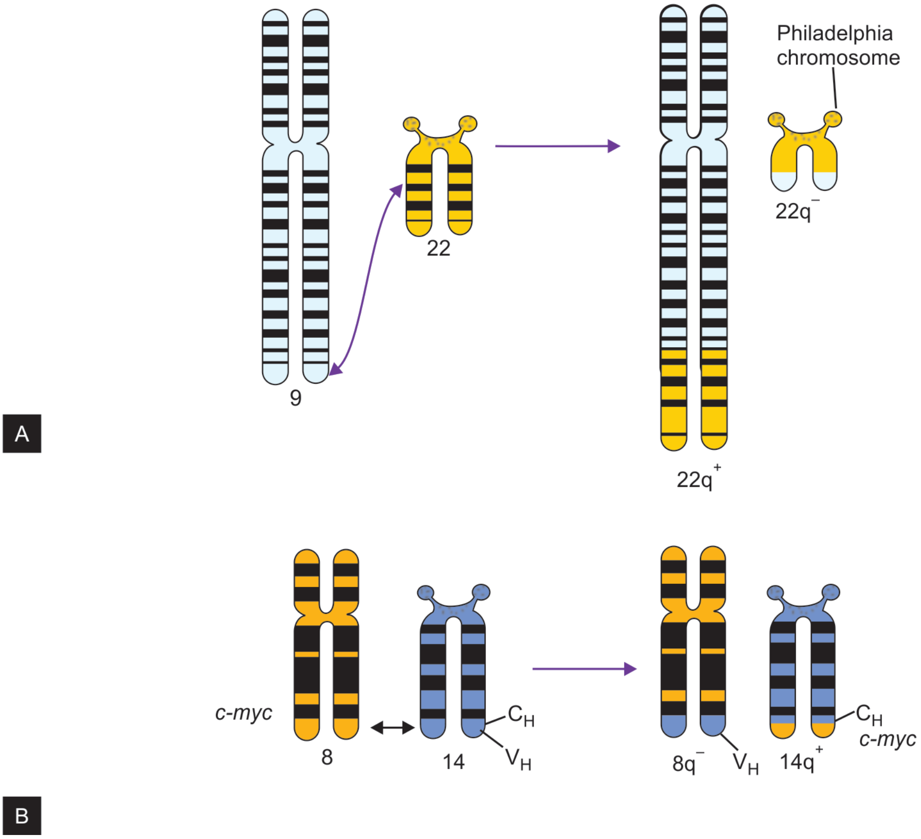
A second set of tumor-associated oncogenes are called antioncogenes or tumor suppressor genes. They encode proteins, which prevent excessive proliferation. Inactivation of these, result in excessive proliferation. The examples of such antioncogenes are *Rb*, $P^{53}$, *DCC, APC, NF1, WT1* (Table 14.1).

A third category of tumor-associated genes regulate apoptosis (programed cell death). The example of this gene is *bcl-2*, which encodes proteins, which either block or induce apoptosis.

## Conversion of Proto-oncogenes to Oncogenes

Huebner RJ and Todardo GJ in 1972 suggested that mutation or genetic rearrangement of proto-oncogenes by carcinogens or virus might alter, normally regulated function converting the proto-oncogenes to oncogenes. It has been seen that some malignant cells contain multiple copies of cellular oncogenes resulting in increased production of oncogenic products.

Besides some cancer cells exhibit chromosomal translocation (movement of proto-oncogenes) from 1 chromosome to other. In cells of the Burkitt's lymphoma, c-myc moves from normal position (chromosome 8) to a



**Figs 14.1A and B:** Chromosomal translocations. **A.** Chronic myelogenous leukemia (CML); **B.** Burkitt's lymphoma. Leukemic cells from all patients with CML contain the so-called philadelphia chromosome, which results from a translocation between chromosomes 9 and 22. Cancer cells from some patients with Burkitt's lymphoma exhibit a translocation that moves part of chromosome 8 to 14. It is now known that this translocation involves c-myc, a cellular oncogene. Abnormalities such as these are detected by banding analysis of metaphase chromosomes. Normal chromosomes are shown on the left and translocated chromosomes on the right.

**Table 14.1** Functional classification of cancer-associated genes

| Type/Name | Nature of gene product |
|---|---|
| **Category I: Genes that induce cellular proliferation** | |
| Growth factors | |
| *Sis* | A form of platelet-derived growth factor (PDGF) |
| Growth factor receptors | |
| *fms* | Receptor for colony-stimulating factor 1 (CSF-1) |
| *ErbB* | Receptor for epidermal growth factor (EGF) |
| *neu* | Protein (HER-2) related to EGF receptor |
| *ErbA* | Receptor for thyroid hormone |
| Signal transducers | |
| *src* | Tyrosine kinase |
| *abl* | Tyrosine kinase |
| *Ha-ras* | GTP-binding protein with GTPase activity |
| *N-ras* | GTP-binding protein with GTPase activity |
| *K-ras* | GTP-binding protein with GTPase activity |
| Transcription factors | |
| *jun* | Component of transcription factor AP1 |
| *fos* | Component of transcription factor AP1 |
| *myc* | DNA-binding protein |
| **Category II: Tumor-suppressor genes, inhibitors of cellular proliferation** | |
| *Rb* | Suppressor of retinoblastoma |
| *p53* | Nuclear phosphoprotein that inhibits formation of small-cell lung cancer and colon cancers |
| *DCC* | Suppressor of colon carcinoma |
| *APC* | Suppressor of adenomatous polyposis |
| *NF1* | Suppressor of neurofibromatosis |
| *WT1* | Suppressor of Wilms' tumor |
| **Category III: Genes that regulate programed cell death** | |
| *Bcl-2* | Suppressor of apoptosis |

position near the immunoglobulin heavy chain enhancer or chromosome 14 may be a major mechanism, how chemical carcinogens and irradiation convert a proto-oncogenes into cancer-inducing oncogene. For instance, a single point mutation in c-ras has been detected in several human cancers such as bladder, colon, lung, etc.

Viral integration into the host cell genome may itself help to convert proto-oncogenes to cancer-inducing oncogenes. A variety of tumors have been shown to express a number of growth factors and a number of growth factor receptors. Expression of receptors for epidermal growth factor has been shown to be amplified in many cancer cells. The epidermal growth factor has been encoded by c-erbB (Table 14.1).

## TUMOR ANTIGENS

Many human tumors express antigens that can induce and be the target of cellular and humoral responses. The relevant tumor antigens fall into two major categories:

1. Unique tumor-specific antigens [tumor-specific transplantation antigen (TSTA)], which are found only in tumor cells and not present in normal cell and therefore represent ideal targets for an immunologic attack.
2. Tumor-associated determinants [tumor-associated transplantation antigen (TATA)], which are found in tumor cells and also in some normal cells. Qualitative and quantitative difference in antigen expression only differentiates tumor cells from normal cells.

A wide variety of cellular proteins have now been identified to function as tumor antigens. The most important of all the molecular mechanisms is the production of a new protein that would occur following infection with potentially oncogenic viruses such as Epstein-Barr virus (EBV), human T-lymphotropic virus (HTLV) or human papillomavirus (HPV). The other mechanism being point mutation or gene rearrangements affecting cellular oncogenes can result in the expression of new epitopes recognizable by the immune system (ras and p53 in breast and colon carcinoma, bcr-abl in chronic myelogenous leukemia): unique tumor antigens can also result from random mutation.

Non-unique proteins, sometimes may serve as a tumor antigen and can result from aberrant expression of fetal or increased expression of differentiation antigen, such as that observed with the expression on human gastric carcinoma cells of ABO blood group antigens disparate from the host ABO blood type or of the melanoma-associated gene (MAGE) antigens in human melanoma cells.

### Unique Tumor-specific Antigen (Tumor-specific Transplantation Antigen)

Tumor-specific transplantation antigens are unique to tumor cells and do not occur in normal cells in the body. They may result from mutation in the cell leading to production of altered cellular protein. Cytosolic processing of these proteins give rise to novel small peptides, which in combination with major histocompatibility cells (MHC I) molecules induce cell-mediated immune response by cytolytic CD8$^+$ cells.

Tumor-specific antigens (TSAs) have been identified on tumors induced with physical or chemical carcinogens and on some virally induced tumors. Methylcholanthrene and ultraviolet light are two carcinogens that have been used extensively to generate lines of tumor cells. Spontaneous tumors, many of which might have been induced by environmental carcinogens, but do not have predictable antigenic markers, which posed problems. Recently improved methods have been used to detect and recover low frequencies tumor reactive T cells and antibodies from the blood, draining lymph nodes and tumor masses of the patient.

Many tumors linked epidemiologically with viruses, viral genomes present in the tumor cells have been isolated, viral proteins expressed in human tumors identified. The antigens appear in tumor cells demonstrated to be potentially immunogenic. Burkitt's lymphoma cells in human, have shown to express a nuclear antigen of EB virus. E6 and E7 protein of HPV are invariably found in more than 80 percent of cases of cervical cancers—a clearest example of tumor-associated antigen.

## Tumor-associated Transplantation Antigens

Unlike tumor-specific transplanted antigens, tumor-associated-transplanted antigens are not unique to tumor cells. They are also present in normal cells, these antigens may be proteins usually expressed only on fetal cells, but not in normal adult cells or they may be protein expressed at low levels by normal cells, but at much higher level in tumor cells. The later category includes growth factors and growth factor receptors, as well as oncogene encoded proteins.

With the development of technologies to produce and screen monoclonal antibodies generated in mice in response to human tumor, the identification of tumor-associated antigens have been possible in last decade. Many of the identifiable antigens are already invaluable diagnostically from distinguishing transformed from non-transformed cells and for defining the cell lineage of transformed cells. Many tumor-associated antigens may be attractive for targeting therapeutic attack.

The various tumor-associated-transplanted antigens are given below.

### *Oncofetal Antigens*

*Carcinoembryonic antigen (CEA):* It is a glycoprotein found on fetal gut and human colon cancer cells, but not on normal adult colon cells. Besides its elevated level in the serum of patients with colorectal cancer, may be found in other inflammatory lesions involving cells of the endodermal origin, such as colitis or pancreatitis as well as patients with other tumors such as pancreatic and breast cancer. Recent studies have suggested that T cell response can be elicited to CEA. Despite the limitations that they are found in so many conditions, monitoring the fall and rise of CEA levels in colon cancer patients, undergoing therapy has proven useful for predicting tumor progression and response to treatment.

*α-fetoprotein:* This is an α-globulin, normally secreted by fetal liver and yolk sac cells and is found in the serum of patients with liver and germinal cell tumor. As it is normally present in adult of non-cancerous state up to certain level, its presence in serum is not diagnostic of tumor, rather serves to monitor tumor growth.

### *Melanoma-associated Antigens*

Several TATA have been identified on human melanomas. Five of these are oncofetal type antigens, (MAGE-1, MAGE-2, etc). They are expressed on other human tumors, but not on normal differentiated tissues except for the testis, where it is expressed on germline cells.

*Cancer testis antigens:* They are tumor-associated proteins detected in malignant cells and germinal tissue.

*Membrane glycoprotein and glycolipid antigens isolated from malignant melanoma:* These antigens are relatively specific for this tumor though, at times, expressed in normal cells such as neuronal tissues.

*Common acute lymphocytic leukemia antigens (CALLA or CD10):* This antigen has been detected in human leukemia cells. This is also detected in low level in granulocytes and kidney.

Besides the tumor-associated antigens, stated above, various other proteins such as oncogene proteins are expressed on tumor cells. For example, human breast cancer cells exhibit elevated expression of the oncogene encoded neu protein, a growth factor receptor, where as, normal adult cells express only a trace amount.

## Immune Surveillance Theory

Immune surveillance theory was first put forwarded by Paul Ehrlich in 1900. He opined that cancer cells are produced in the body and are recognized as foreign by the immune system. 50 years later, Lewis Thomas suggested that cell-mediated limb of the immune response patrol the body and eliminate the cancer cells. According to these concepts, tumor occurs only, when there is a failure in immunosurveillance and the cancer cells escape the immune attack. Escape from immunosurveillance may be due to reduction in expression of tumor antigen or by an impairment of immune response to these cells.

Amongst the early observation, to support the immunosurveillance theory, there is increase in the incidence of tumor in transplantaion patients with immunosuppressive drugs. But other findings object to this theory of increased incidence of tumors in immunosuppressive therapy. In nude mice (without-thymus), where there is T cell deficiency; there should be more incidence of cancer, but it does not happen. The incidence of cancer is not more than the normal mice. Further more, although in the individuals with immunosuppressive drug therapy, the incidence of cancer of immune system is more, the incidence of other cancers (breast, colon and lung) is not increased.

It has been seen that animals injected with very high doses or low doses of tumor cells develop tumor, but with intermediate doses there is no tumor formation. The mechanism by which a low dose of tumor cells sneaks through is difficult to reconcile the immune surveillance theory. Finally, this theory assumes that there is only qualitative difference in the cancer cells and normal cells. Infact, as stated earlier, many types of tumors do not express TSAs, any immune response that will develop must be induced by quantitative difference in antigen expression in normal cells and tumor cells. However in virus-induced tumor, there is expression of virus related antigen against which the immune system will react.

Nevertheless, apart from the tumors caused by viruses, the basic concept of immune surveillance theory is that the malignant tumor arises only when there is impairment of immune system or when the tumor cell loses its immunogenicity enabling them to escape immunosurveillance, at this time, remains unproved. Inspite of this, it is clear that an immune response can be generated to tumor cells and therapeutic approaches can be aimed at increasing that response to combat malignant cells.

## Immune Response to Tumor

### *Natural Immunity*

Natural immunity to tumors is mediated by activated macrophages, neutrophils and natural killer cells (NK cells). Their action may be cytolytic or cytostatic inhibiting tumor growth. This type of immunity does not require antibodies and displays no antibody specificity (Fig. 14.2).

### *Adaptive Immunity*

When tumor antigens are introduced into experimental animals, it is seen that both, humoral immunity and cell-mediated immunity play important roles in causing destruction of the tumor cells. Similar things happen in human being.

There are two major mechanisms by which antibodies may mediate tumor cell lysis. Complement fixing antibodies bind to tumor cell membrane and promote attachment of complement components ultimately creating pores on tumor cell membrane, resulting in cell disruption. An alternative mechanism is antibody-dependent cell-mediated cytotoxicity (ADCC) in which antibodies, usually of the class immunoglobulin G (IgG), form an

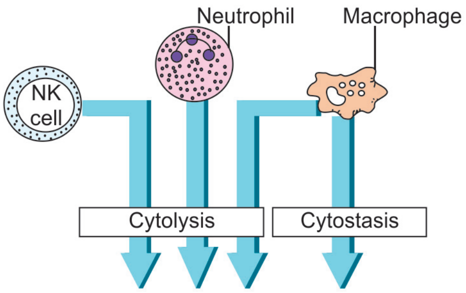


**Fig. 14.2:** Mode of action of various cells to tumor immunity

intercellular bridge by binding via the variable region to a specific determinant on target cell and via the fragment crystallizable (Fc) region to effector cell expressing Fc receptors. Many potential effector cells include NK cells, macrophages and granulocytes. ADCC mechanism may be more important as *in vivo* effector mechanism than complement-mediated lysis.

T cell response is most important immune response for the control of growth of antigenic tumor cells. T cell immunity to tumor, reflects the function of two subsets of lymphocytes. The first subset is the MHC II restricted $CD4^+$ T helper ($T_h$) cell that mediate its effects by direct interaction with antigen-presensting cells (APC) and by secretion of lymphokines to activate other effector cells and induces inflammatory response. The second subset is MHC I restricted $CD8^+$ cytotoxic T cells ($T_c$) that can mediate their effects by direct lytic action on tumor cells, though they also produce lymphokines. Most tumor cells express class I, but not class II molecules so that the $CD4^+$ ($T_h$) cannot directly recognize tumor cells. The $T_h$ cells depend on APC such as dendritic cells or macrophages to present the relevant tumor antigens in the context of class II molecules for activation. The T cells further activate dendritic cells and secrete lymphokines that activate $T_c$ cells, macrophages, NK cells and B cells.

T cells also produce tumor necrosis factor-beta (TNF-β) which may be directly lytic to tumor cells. In contrast, the $T_c$ cells have got direct lytic effect on tumor cells, killing tumor cells by disrupting the target membrane and disintegrating the nucleus. Therefore, effective $T_c$ cell response is dependent upon the class II restricted $T_h$ cells cooperation for necessary activation and proliferation of $T_c$ cells through interleukin-2 (IL-2). Recent studies have shown that some tumor cells have defective antigen processing machinery with the results that class I molecules do not get loaded with peptides and transported to the surface, class I expression is low and even potentially highly immunogenic tumor antigen cannot be presented to the immune system.

Natural killer cells are not MHC restricted. Hence, the diminished expression of MHC I in tumor cells does not affect the killing and destruction of tumor cells by NK cells. The precise nature of the activating signal for tumor cell is less certain, but a variety of molecules such as CD48 and surface glycoproteins are candidates. Cytolysis by NK cells is mediated by the release of cytotoxic factor and the use of perforins to puncture holes in target cell membrane. The cytotoxic action of the NK cells can be augmented both *in vitro* and *in vivo* with lymphokines IL-2 and interferon-γ (IFN-γ) (Fig. 14.3) and via cross-linking the activating Fc receptor. Thus, NK activity can be amplified by both immune T cell and B cell responses. Recent studies reveal that augmentation of NK cell activity in visceral organs, enhances resistance to the growth of metastases.

Lymphokine-activated killer (LAK) cells are additional cytotoxic cells have resemblance with NK cell. LAK cells can be induced by very high pharmacological doses of IL-2 and kill much broad spectrum of tumor targets than NK cells (Fig. 14.3). NKT cells are another class of effector cells, which express both NK and T cell markers and appear to be activated by non-classical class I molecules.

Macrophages serve two purposes. They serve as APCs and also effector cells. The resting macrophages are not cytolytic to tumor cells *in vitro*, but can be cytolytic with macrophage activating factors (MAF). MAF INF-γ, TNF-β, IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are commonly secreted by T cells following antigen specific stimulation. There are three possibilities, how the activated macrophages bring about destruction of tumor cells. First, activated macrophages bind with antibody coated tumor cells through Fc receptor and kill by ADCC mechanism. Secondly, the antitumor activity of the activated macrophages are probably mediated by lytic enzymes, reactive oxygen and nitrogen intermediates. Last, but not the least, the activated macrophages liberate TNF-α, which has also got direct antitumor activity on tumors.

### *Immunological Escape*

The ability of a tumor to escape from immunological control may depend upon a balance between the effectiveness of the immune system and a variety of factors promoting escape. The factors are given below.

*Sneaking through (tumor kinetics):* Tumor cells administered sufficiently in low doses develop into cancer, while greater doses are rejected. Therefore, tumor cells may sneak through and not be recognized until growth is established.

*Antigenic modulation:* In the presence of antibody, some antigens are modulated of the cell surface. This involves antigen shedding, endocytosis and redistribution within the membrane without a complete loss of determinant from the cell surface. Antigenic modulation facilitates escape by removing target antigens that the immune system effector cells would recognize.

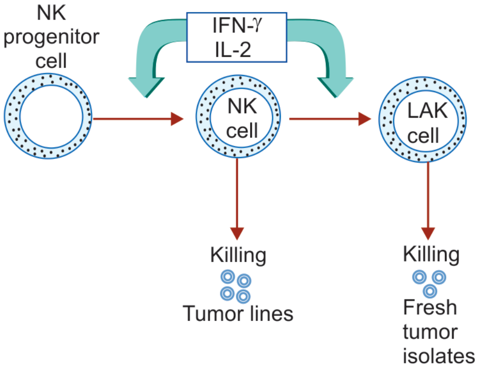


**Fig. 14.3:** Tumor immunity by natural killer (NK) cell and lymphokine-activated killer (LAK) cell

*Antigen masking:* Certain molecules such as sialomucin, which are frequently bound to the surface of the tumor cells, mask tumor antigens and prevent adhesion of attacking lymphocytes. Masking can be overcome by the treatment, which degrades sialomucin. For example, with *Vibrio cholerae* neuraminidase.

*Blocking factors:* Soluble tumor antigens compromise the expression of T immunity by saturation of antigen-binding sites, particularly in the tumor environment, where the concentration of the shed antigens likely to be the highest. Antibodies and antigen-antibody complex can block the cytotoxicity of the host lymphocytes. More probably there is block of Fc receptors on LAK cells.

*Genetic factors:* The immune response is under genetic control. The difference in immune response to tumor antigen, shown by different individuals in a species is determined by genetic differences. Immune response *(Ir)* genes controls this property.

*Reduction of class I MHC molecules:* Malignant transformation of cells is often associated with a reduction or complete loss of class I MHC molecule. The decrease in MHC I expression on cells is associated with progressive tumor growth. So the absence of MHC molecules, on a tumor is generally indication of poor prognosis.

*Lack of costimulatory signals:* T cell activation requires an activating signal, triggered by MHC I peptide complex with T cell receptor and a costimulation signal triggered by B7 molecule on the APC and CD28 on T cells. Both signals are needed for IL-2 production and proliferation of T cells. In the absence of costimulatory signals there is anergy (immune tolerance).

*Tumor products:* The subversion of immune response by products of tumors other than antigen can also be envisaged. Prostaglandins and negatively regulated NK and K cells function constitutively. Similarly, other humoral factors act non-specifically to impair inflammatory response, chemotaxis and the complement cascade or to augment the formation of blood supply within solid tumors.

*Growth factors:* Amplification of T cell responses is critically dependent on the availability in the production of IL-1 by macrophages or in the degree or cooperation between the T cell subsets or in the availability of IL-2 could conceivably limit the overall responses to a tumor (Fig. 14.4).

## Cancer Immunotherapy

Although various immune responses can be generated to tumor cells, they may not be sufficient to prevent tumor growth. One aspect to prevent cancer is to increase the natural defenses by various approaches. Several types of cancer immunotherapies, in current use or underdevelopment are described.

### *Biological Response Modifiers*

Biological response modifiers could be used to activate NK cells and APCs. These include attenuated strains of *Mycobacterium bovis* called bacille Calmette-Guérin (BCG), *Corynebacterium parvum.* These adjuvants activate macrophages; increasing their expression with various cytokines, class II MHC molecules and the B7 costimulatory molecule. IFN-γ and IL-2 also activate NK cell and macrophage activities.

### *Cytokine Therapy*

Large-scale production of cytokines has been possible due to cloning the various cytokine genes. A number of experimental and clinical approaches have been made to use recombinant cytokines, to augment the immune response against cancer. Among the cytokines, which have been evaluated are IFN-α, β and γ; IL-1, IL-2, IL-4, IL-5, IL-12, GM-CSF, TNF. Many obstacles come on the way. The notable obstacle is the complexity of the cytokine network itself. Some cytokines act antagonistically.

Today, the most clinical trials have been done on IFN-α. Daily injection of recombinant IFN-α have been shown to induce partial or complete regression in some of the hematological malignancies such as leukemia, lymphomas, myelomas and with solid tumor such as melanoma, Kaposi's sarcoma, renal and breast cancers.

Interferons cause antitumor activity in three ways:

1. All three types of interferon increase class I MHC expression in tumor cells.
2. IFN-γ also causes increased expression of MHC II molecules on macrophages. When the MHC I expression in tumor cells and MHC II expression on macrophages increased, the cytotoxic T lymphocytes (CTLs) activity is greatly enhanced.
3. Finally, IFN-γ directly or indirectly increases the activity of CTLs, NK cells and macrophages.

In some instances, it has been seen that both TNF-α and -β have got direct action on tumors killing and checking the

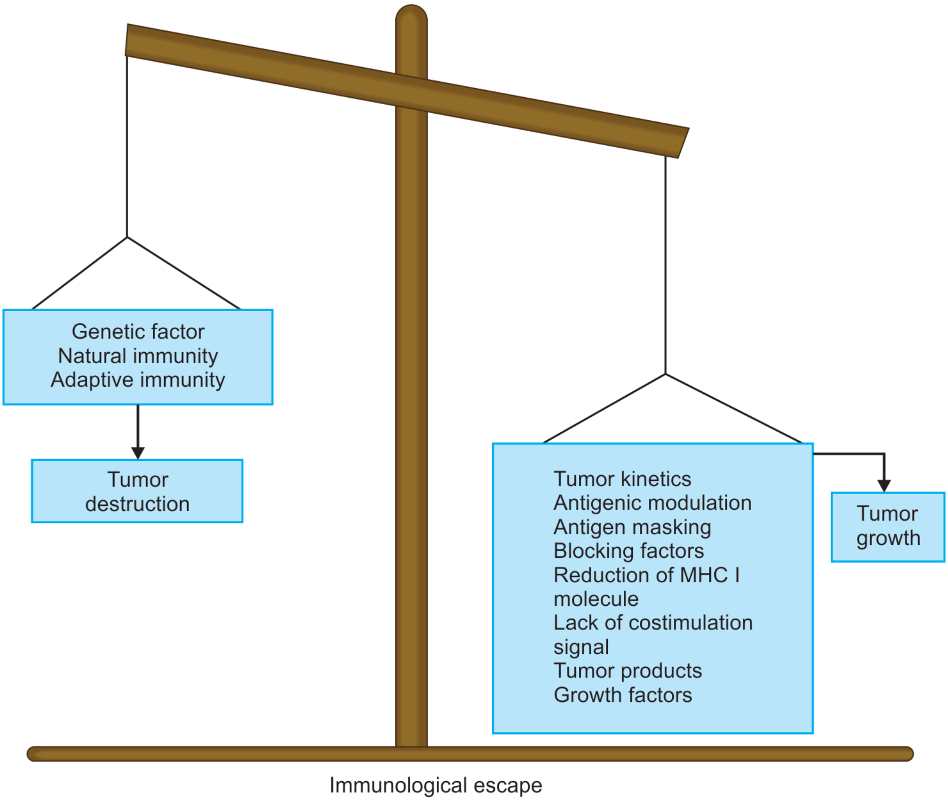

Genetic factor
Natural immunity
Adaptive immunity
Tumor destruction
Tumor kinetics
Antigenic modulation
Antigen masking
Blocking factors
Reduction of MHC I molecule
Lack of costimulation signal
Tumor products
Growth factors
Tumor growth
Immunological escape

**Fig. 14.4:** Immunological escape resulting in tumor

proliferation of tumor cells. TNF-α also inhibits the tumor-induced vascularization by damaging the vascular endothelial cells into vicinity of tumor cell, thus cutting the blood supply to tumor.

### *In Vitro-activated LAK and TIL Cells*

Lymphokine-activated killer cells can be produced by *in vitro* culturing the lymphocytes with X-irradiated tumor cells with IL-2 and tumor antigens. These activated lymphocytes mediate the tumor destruction more than the untreated lymphocytes.

Tumor contains lymphocytes that have infiltrated the site as a part of immune response. By taking small biopsy from the tumors, one can obtain a population of lymphocytes and expand further with IL-2. These lymphocytes are known as activated tumor-infiltrating lymphocytes (TILs). TILs are of interest because of their higher antitumor activity. The expanded populations are injected into autologous patients together with recombinant IL-2. The TILs therapy is of great use in melanoma and renal cell carcinoma.

## MONOCLONAL ANTIBODIES

A number of encouraging results have been obtained with therapy using monoclonal antibodies against tumor-associated antigen and TSAs. Anti-idiotype monoclonal antibodies have been used with some success in treating human B cell lymphoma and T cell leukemia. A more general monoclonal antibody therapy for B cell lymphoma is based on the facts that most B cells, whether normal or cancerous, bear 'B' cell lineage. One such determinant that is CD20 has been the target of intensive efforts. A monoclonal antibody to it, raised in mice, but engineered to contain human sequences has been useful in the treatment of B cell lymphoma. Besides

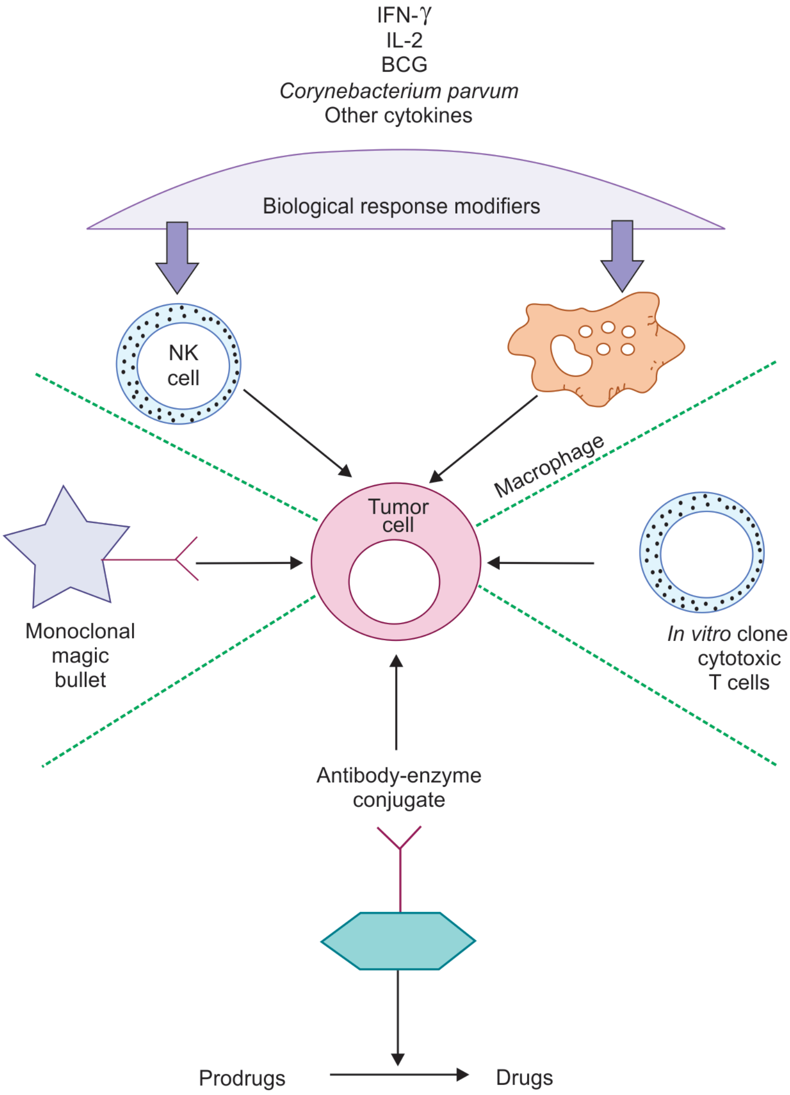

IFN-γ
IL-2
BCG
*Corynebacterium parvum*
Other cytokines
Biological response modifiers
NK cell
Macrophage
Tumor cell
Monoclonal magic bullet
*In vitro* clone of cytotoxic T cells
Antibody-enzyme conjugate
Prodrugs
Drugs

**Fig. 14.5:** Prospects for immunological intervention

CD20, a number of tumor-associated antigens are tested on clinical trials.

A variety of tumors express significantly a number of growth factor receptors, which are promising targets for antitumor monoclonal antibodies. Monoclonal antibodies have also been used to prepare tumor specific antitumor agents. Radioactive isotopes, chemotherapy drugs potent toxins could be tagged with monoclonal antibodies against TSA and tumor-associated antigen. These agents are delivered, specifically to tumor cells without producing delitorious effects on normal cells. Immunotoxins (diphtheria toxin) can be coupled with monoclonal antibody against TSA. These magic bullets can kill tumor cells without affecting normal cells. Use of immunotoxins, specific for tumor antigens in a variety of cancers (colon, breast, melanoma, lymphoid leukemia) has been evaluated in some trials. Against lymphoma and leukemia, this therapy has exhibited partial or complete remission. Alternatively, antibody enzyme conjugates located at the tumor site could act on systemically administered prodrugs to release toxic drugs, selectively at the critical site (Fig. 14.5).

### Cancer Vaccines

The key element in designing vaccine is the identification of significant tumor antigens by genetic and biochemical approaches. The strategy should be the effective presentation of antigen, so that activated helper and CTLs are produced in good number.

In case of few human cancers, which are of viral origin, vaccination against those viruses may be effective. The example of one such virus is HPV, which causes malignant transformation of cells leading to cervical cancer. Vaccines against HPV infection have already evolved and immunization protocol has been used extensively for prevention of cervical cancer. Steps are also being taken to develop vaccines to prevent cancers from recurring in individuals, who have skin cancer (melanoma) and renal carcinoma. Clinical trials have been attempted to prepare vaccines using patient's own tumor cells after surgical removal. Such vaccines are directed to generate immune response against any malignant cells, remaining in the body.

## STUDY QUESTIONS

### Essay Questions

1. In what ways do tumor cells differ antigenically from normal cells? Explain how tumor cells may be destroyed by the immune system.
2. If tumor cells can be destroyed by the immune system, how does cancer develop? What does immunotherapy involve?

### Short Notes

1. Oncogenes.
2. Tumor antigens.
3. Oncofetal antigens.
4. Biological response modifiers.
5. Cancer vaccines.

## SUGGESTED READING

1. Black JG. Microbiology Principles and Applications, 3rd edition. 1996.
2. Borish L. Genetics of allergy and asthama. Ann Allergy Asthma Immunol. 1999;82(5):413-24.
3. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
4. Goldsby RA, Kuby TJ. Immunology, 6th edition. 2007.
5. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
6. Jawetz. Textbook of Microbiology, 25th edition. 2010.
7. Lippincott. Immunology. 1st Indian print. 2008.
8. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. 2006.
9. Stites. Basic and Clinical Immunology, 8th edition. 2007.

# Transplantation Immunology

## 15

Transplantation refers to transfer of tissues or organs from one site to another. The desire to accomplish transplantation arose from realization that the disease can be cured by implantation of healthy organs or tissue from a donor in place of diseased one in the recipient.

Though so many attempts were made earlier, the first successful human kidney transplantation was done between identical twins in 1954. Today transplantation of organs (kidney, pancreas, heart, liver, bone marrow, etc.) are done among non-identical individuals, with ever increasing frequency of success, using various techniques for suppressing the intensity of immune reaction. Human ABO blood group discovery by 'Karl Landsteiner' was a key event in development of successful organ or tissue transplantation, because blood transfusion is a form of transplantation. The ABO system provided a simplified model of the concept that immune recognition of genetically foreign substance plays an important role in graft rejection. Introduction of mismatched blood group to a recipient, who has got antibodies (isoagglutinin) to the donor's blood group antigen, causes immediate often fatal response. Before attempting transplanting organ or tissue from a donor to recipient, the blood grouping of both the donor and recipient is essential, as the vascular endothelium of the grafted tissues also expresses blood group antigens. The donor organ can be attacked by blood group antibodies. Matching other blood group antigens (Rh or Lews, etc.) are not that important, because they are only expressed in the red blood cells (RBCs).

## GRAFT REJECTION

The objective of studying transplantation immunology is to prevent graft rejection between genetically non-identical individuals. Various immunosuppressive agents are being used to diminish the immunological reaction of the grafted tissue. But the problem with the long-term use of these agents is detrimental to the recipient of the graft.

### Types of Graft

Grafts can be categorized as autografts, isografts, allografts and xenografts (Fig. 15.1).

*Autograft*

Autograft is self-tissue, which can be transferred from one site to the other site in the same individual. Autograft is usually performed in burn patients transferring healthy area to burn area. This does not elicit immune response.

*Isograft*

*Isograft* is the tissue transferred between genetically identical individuals (monozygote twins) in human or mice of the same inbred strains. They do not express antigen, foreign to the recipient, hence they do not produce rejection response.

### *Allograft*

*Allograft* is the tissue transferred between genetically two individuals of the same species. In humans, the tissues or organ grafts between two individuals, other than identical twins, fall in this group. In mice, an allograft is done from one inbred strain to another.

### *Xenograft*

*Xenograft* is the transfer of tissue between different species, (e.g. the grafting of monkey kidney to human). Xenograft exhibits the greatest genetic disparity and therefore induces vigorous rejection.

## Specificity and Memory of the Rejected Response

The time of rejection depends on the tissue involved. Skin grafts are rejected quicker than heart and kidney. Irrespective of these time differences, the immune response involving rejection displays the specificity and memory. When a skin graft from a mouse of strain 'A' is grafted in mouse of strain B, rejection occurs. This is known as first set rejection. The skin becomes revascularized and then there is infiltration of lymphocytes, monocytes, neutrophils and other inflammatory cells. There is decreased vascularization and by 10 days, there is visible necrosis. By 14th day, the graft is sloughed out and there is complete rejection. When the mouse of strain B is subjected to a second graft from the mouse of strain 'A', graft rejection develops more quickly with complete rejection occurring within 5 to 6 days. The quick rejection is attributed due to immunological memory.

## Role of Cell-mediated Immunity

T cells take part in allograft rejection (Fig. 15.2). It is seen that nude (thymectomized) mice lack functional T cell and therefore incapable of allograft rejection, even the xenografts rejection. Analysis of the T cell subsets involvement in allograft rejection has revealed, both CD4$^+$ cells and CD8$^+$ cells. It was found that removal of CD8$^+$ cells by monoclonal antibodies had no effect on graft survival and the graft was rejected at the same time as that of control mice. Removal of CD4$^+$ cells by monoclonal antibodies (mAbs) prolonged the survival rate from 15 to 30 days. However, the removal of both CD4$^+$ and CD8$^+$ cells by their respective mAbs resulted in long-term survival of the allograft (up to 60 days). From this study, it was inferred that both CD4$^+$ and CD8$^+$ cells that participate in

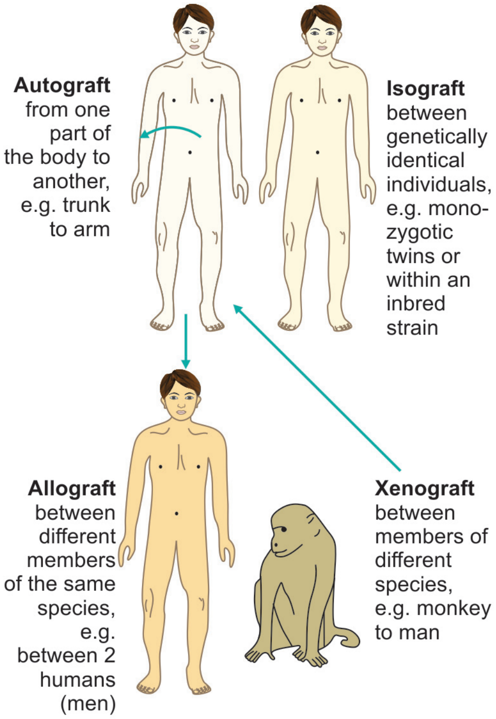

**Autograft**
from one part of the body to another, e.g. trunk to arm
**Isograft**
between genetically identical individuals, e.g. monozygotic twins or within an inbred strain
**Allograft**
between different members of the same species, e.g. between 2 humans (men)
**Xenograft**
between members of different species, e.g. monkey to man

**Fig. 15.1:** Genetic barriers to transplantation. The genetic relationship between the donor and recipient determines whether the rejection will occur. Autografts or isografts are usually accepted, while allografts and xenografts are not.

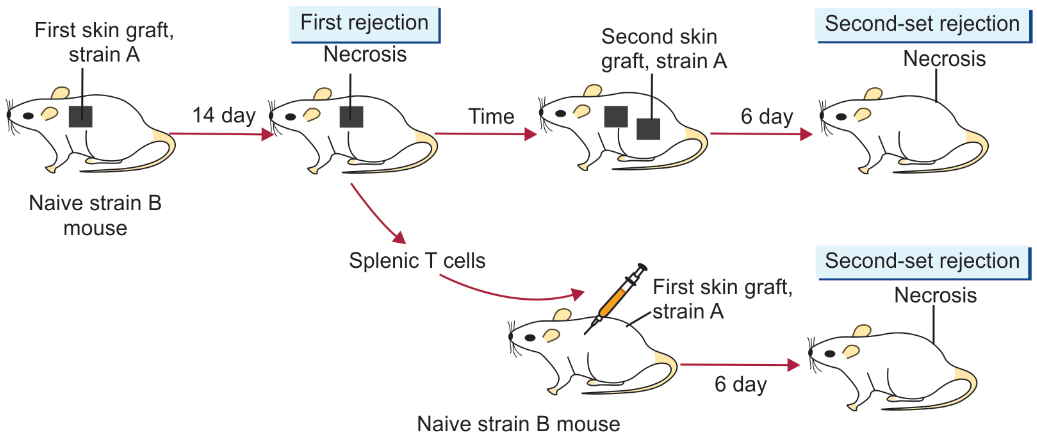


**Fig. 15.2:** Experimental demonstration that T cells can transfer allograft rejection. When T cell derived from an allograft-primed mouse are transferred to an unprimed syngeneic mouse, the recipient mounts a second-set rejection to an initial allograft from the original allogeneic strain.

the rejection and their collaboration potentiate the rejection.

## Transplantation Antigens

Tissues, which are antigenically similar are known as histocompatible antigens. Such tissues do not evoke immunological response, hence there is no rejection of tissue. Tissues, which display significant antigenic difference, evoke immune response, hence induce rejection. These antigens are known as histoincompatible antigens. The various antigens, which determine histocompatibility are encoded by more than 40 different loci, but the loci responsible for more vigorous allograft rejection reactions are located within major histocompatible complex (MHC) (Table 15.1). The organization of MHC in mice and human being are H-2 complex and human leukocyte antigen (HLA) complex respectively. MHC loci are inherited as complete set called haplotype from each parent (Figs 15.3A and B).

Within an inbred strain, all animals are homozygous at each MHC locus. For example, when mice from two different inbred strains, with haplotype b and R are mated, all the F1 progeny inherit one haplotype from each parent (refer Fig. 15.3A). These F1 offspring have the MHC type b/R and can accept grafts from either parent. Transplantation in the reverse direction (from F1 to parent) will not succeed because each parent lacks one of the F1 haplotype. While transplantation between members of inbred strain of animals is successful, an exception is seen when the donor is male and the recipient, a female. Such grafts are rejected as the grafted male tissue (XY) will have antigens determined by Y chromosome, which will be absent in the female (XX) recipient. Graft from female to male will have no problem. This unilateral sex-linked histoincompatibility is known as Eichwald-Silmser effect.

Major histocompatible complex identity of donor and host is not the sole factor determining tissue acceptance, when the tissue

**Table 15.1** Simplified organization of the major histocompatibilty complex (MHC) in the mouse and human

| **Feature** | **Comparison** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Mouse H-2 complex** | | | | | | | | |
| Complex | H-2 | | | | | | | |
| MHC class | I | II | | III | | I | | |
| Region | k | IA | IE | S | | D | | |
| Gene products | H-2K | IA<br>αβ | IE<br>αβ | C proteins | TNF-α<br>TNF-β | H-2D | H-2L | |
| **Human MHC complex** | | | | | | | | |
| Complex | HLA | | | | | | | |
| MHC class | II | | | III | | I | | |
| Region | DP | II | | C4, C2, BF | | B | C | A |
| Gene products | DP<br>αβ | DQ<br>αβ | DR<br>αβ | C proteins | TNF-α<br>TNF-β | HLA-B | HLA-C | HLA-A |

The MHC is referred to as the H-2 complex in mice and as the HLA complex in humans. In both species the MHC is organized into a number of regions encoding class I, class II and class III gene products. The class I and class II gene products shown in this table are considered to be the classical MHC molecules. The class III gene products include complement (C’) proteins and the tumor necrosis factors (TNF-α and -β).

is transplanted between genetically different individuals, even if their MHC antigens are identical, the transplanted tissue can be rejected because of differences in various minor histocompatibility loci. The tissue rejection induced by minor histocompatibility difference is usually less vigorous than that induced by major histocompatibility differences. For this reason, successful transplantation requires immunosuppression even if the donor and the recipient are HLA identical.

## FACTORS FAVORING ALLOGRAFT SURVIVAL

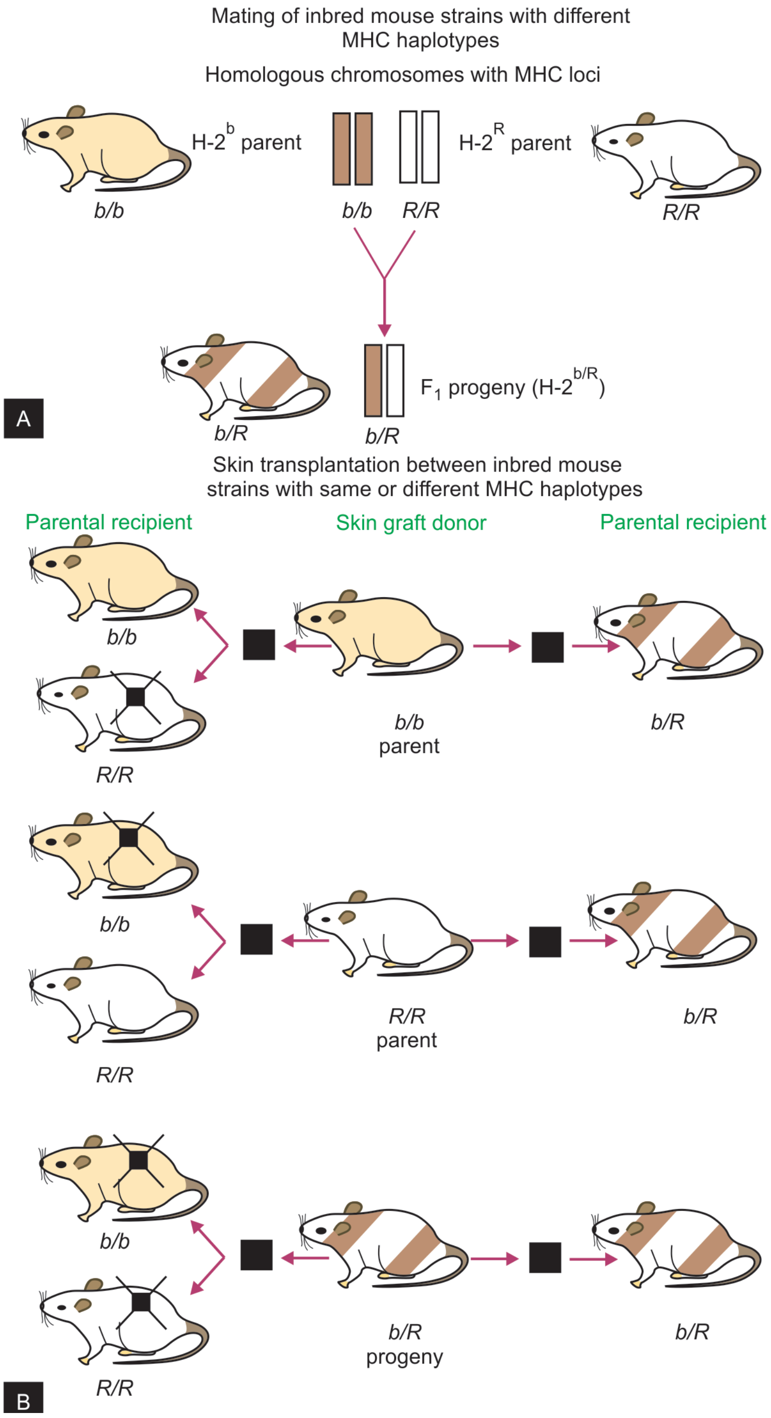
### ABO Blood Group Compatibility

The blood group antigens are expressed on RBCs, epithelial cells and endothelial cells. Antibodies produced in the recipient to any of these antigens, that are present on transplanted tissue, will induce complement-mediated lysis of the incompatible donor cells.

### Human Leukocyte Antigen Typing of Potential Donors and Recipient

*Microcytotoxicity Test*

In this test, white blood cells (WBCs) from the potential donors and recipient, are placed in wells in a microtiter plate and then antibody specific for various class I and class II are added to different wells. The microtiter plate is incubated and then complement is added. Microcytotoxicity is assessed by the uptake or exclusion of various dyes (trypan blue, eosin) by the cells. If the WBCs express MHC allele for which particular mAb is specific, then lysis of the cells occur. Then these dead cells will take up dye such as trypan blue.


Mating of inbred mouse strains with different MHC haplotypes
Homologous chromosomes with MHC loci
H-2b parent
H-2R parent
b/b
b/b
R/R
R/R
b/R
b/R
F1 progeny (H-2b/R)
A
Skin transplantation between inbred mouse strains with same or different MHC haplotypes
Parental recipient
Skin graft donor
Parental recipient
b/b
R/R
b/b parent
b/R
b/b
R/R
R/R parent
b/R
b/b
R/R
b/R progeny
b/R
B

**Figs 15.3A and B:** Mating and skin transplantation of inbrd mouse. **A.** Illustration of inheritance of MHC haplotypes in inbred mouse strains. The letters b/b designate a mouse homozygous for the H-$2^b$ MHC haplotype, R/R homozygous for the H-$2^R$ haplotype and b/R a heterozygote. Because the MHC loci are closely linked and inherited as a set, the MHC haplotype of $F_1$ progeny from the mating of two different inbred strains can be predicted easily; **B.** Acceptance or rejection of skin grafts is controlled by the MHC type of the inbred mice. The progeny of the cross between two inbred strains with different MHC haplotypes (H-$2^b$ and H-$2^R$) will express both haplotypes (H-$2^{b/R}$) and will accept grafts from either parent and from one another. Neither parent strain will accept grafts from the offspring.

HLA typing based on antibody-mediated cytotoxicity indicates the presence or absence of various MHC alleles. Antisera for HLA typing were originally obtained from multigravidae, placental fluid and from multiple blood transfusion recipients. These are now being replaced by mAbs (Figs 15.4A and B).

### *Mixed Lymphocytic Reaction*

In this test, the lymphocytes from the potential donor is mixed with the lymphocytes of the recipient. Prior to mixing, the lymphocytes for the potential donor are X-irradiated or treated with mitomycin 'C'. Hence, the donor lymphocytes act as stimulator and the recipient lymphocyte as responder cells. Proliferation of recipient T cells, which indicates T cell activation, is measured by the uptake of 3H-thymidine. Greater the activation of recipient lymphocytes more would be the 3H-thymidine uptake. Lesser the MHC II incompatibility, intense is the proliferation of recipient lymphocytes. The prognosis of the survival of the graft is poor (Fig. 15.4C).

### *Molecular Methods*

Molecular methods have been developed for tissue typing. These include restriction fragment length polymorphism (RFLP) with Southern blotting and polymerase chain reaction (PCR) amplification using sequence specific primers.

### *Immunosuppression*

Despite a perfect match in MHC molecules in the graft between the donor and recipient, there may be also rejection. The rejection may be attributable to the immune reaction against minor histocompatibility antigens. Immunosuppression can be achieved, in animals, by neonatal thymectomy and administration of antilymphocyte serum. In man immunosuppressive drugs are given to suppress cell-mediated immunity (CMI). Steroids, azathioprine and fungal metabolites (cyclosporin A) are effective agents causing immunosuppression.

## TRANSPLANTS TO IMMUNOLOGICALLY PRIVILEGED SITES

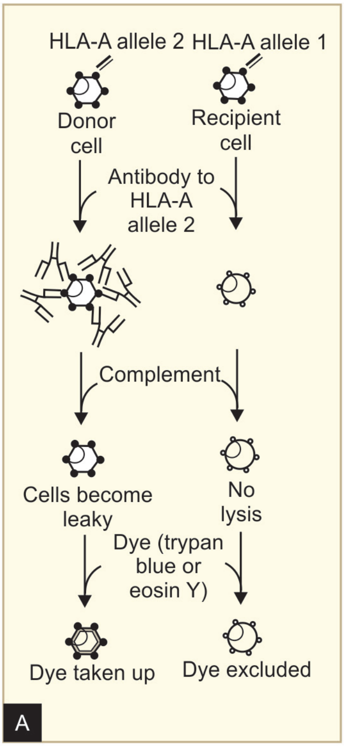
Immunologically privileged sites are the areas, where the allografts can be placed without a rejection reaction. Immunologically privileged sites fail to induce an immune response, because they are effectively sequestered (hidden) from the cells of the immune system. These sites include anterior chamber of the eye, cornea, uterus, testis, brain and the cartilages. Each of these sites are characterized by the absence of lymphatic vessels and in some cases, absence of blood vessels. Consequently, the alloantigens of the grafts are not able to sensitize the recipients lymphocytes. Therefore, there is an increase likelihood of acceptance even if the antigens are not matched.

Fetus can be considered as an intrauterine allograft as it contains antigens (fetus, which is a part of father), which are foreign to mother. The reason is not clearly known, but however, there are certain explanations in favor of acceptance of the graft. They are:

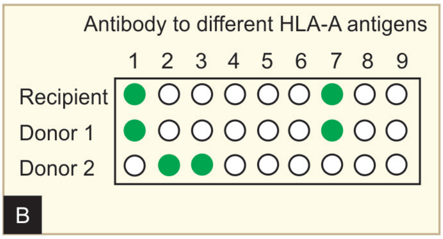
1. Placenta produces certain hormones, which are locally immunosuppressive.
2. The trophoblastic cell layers of the placenta, which separates fetus from mother, lack of major histocompatibility antigens (MHC molecules, both class I and II) or inadequately expressed, which cannot generate immune response.
3. Antigen shedding by fetus blocks the aggressive T cells or antibodies by an enhancement effect.
4. An incomplete mucopolysaccharide barriers, rich in sialic acid, surrounds the trophoblastic cells protecting them from cytotoxic lymphocytes.

### Mechanism Involved in Graft Rejection

Cell-mediated immune response plays principal role in rejecting the graft (alloantigens

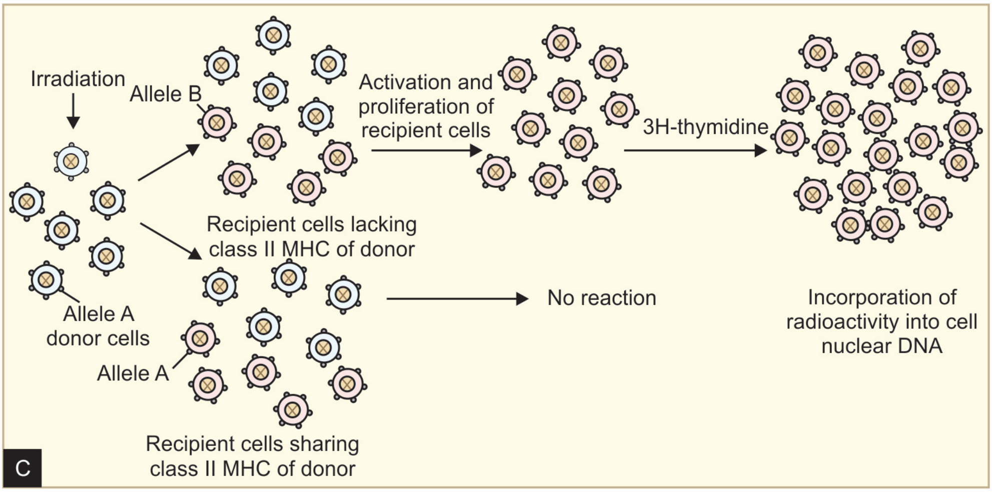

HLA-A allele 2
HLA-A allele 1
Donor cell
Recipient cell
Antibody to HLA-A allele 2
Complement
Cells become leaky
No lysis
Dye (trypan blue or eosin Y)
Dye taken up
Dye excluded
A

**Figs 15.4A to C:** Typing procedures for HLA antigens (A, B). HLA typing by microcytotoxicity. **A.** White blood cells from potential donors and the recipient are added to separate wells of a microtiter plate. The example, depicts the reaction of donor and recipient cells with a single antibody directed against an HLA-A antigen. The reaction sequence shows that, if the antigen is present on the lymphocytes, addition of complement will cause them to become porous and unable to exclude the added dye; **B.** Because cells express numerous HLA antigens, they are tested separately with a battery of antibodies specific for various HLA-A antigens. Here, donor 1 shares HLA-A antigens recognized by antisera in wells 1 and 7 with the recipient, whereas donor 2 has none of HLA-A antigens in common with the recipient; **C.** Mixed lymphocytes reaction to determine identity of class II HLA antigens between a potential donor and recipient. (Contd...)



Antibody to different HLA-A antigens

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Recipient | ● | ○ | ○ | ○ | ○ | ○ | ● | ○ | ○ |
| Donor 1 | ● | ○ | ○ | ○ | ○ | ○ | ● | ○ | ○ |
| Donor 2 | ○ | ● | ● | ○ | ○ | ○ | ○ | ○ | ○ |


Irradiation
Allele B
Activation and proliferation of recipient cells
3H-thymidine
Recipient cells lacking class II MHC of donor
Allele A donor cells
No reaction
Incorporation of radioactivity into cell nuclear DNA
Allele A
Recipient cells sharing class II MHC of donor
C

(Contd...) Lymphocytes from the donor are irradiated or treated with mitomycin C to prevent cell division and then added to cell from the recipient. If the class II antigens on the two populations are different, the recipient cells will divide rapidly and will take up large quantities of radioactive nucleotides into the newly synthesized nuclear DNA. The amount of radioactive nucleotide uptake is roughly proportionate to the MHC class II differences between the donor and recipient lymphocytes.

primarily MHC molecule). Both delayed hypersensitivity reaction ($T_h1$-mediated) and cell-mediated cytotoxicity reaction (CTL-mediated) are involved in the rejection. The process of graft rejection can be divided into:

1. Sensitization phase in which there is sensitization and proliferation of reactive lymphocytes.
2. An effector stage in which immune destruction of the graft takes place.

### *Sensitization Stage*

In response to alloantigens present in the graft, both $CD4^+$ and $CD8^+$ cells proliferate. Both major and minor histocompatibility antigens can be recognized. But the minor histocompatibility antigens are weak and do not produce vigorous rejection. The response to major histocompatibility antigens involves also the association of MHC molecules with cleaved peptides (processed antigens). The peptides present in the groove of MHC I molecule, for $CD8^+$ cells response, are derived from protein synthesized from allogeneic cells of the graft tissue. The peptides present in the groove of MHC II molecule, for CD4 response, are proteins taken up and processed through the endocytic pathway of the allogeneic antigen-presenting cell (APC). At times, peptide fragments meant for allogeneic class molecule, can be presented within the groove of a class II molecule.

The T helper cells ($T_h$ cells) of the host are activated only when the APC expresses appropriate antigenic ligand—MHC II complex along with costimulatory signals. Dendritic cells are the vital APCs because of their presence in majority tissues, as well as high expression of MHC II molecules. APCs of host origin can also enter the grafted tissue, endocytosed the alloantigens (both major and minor histocompatibility molecules) and present them as processed peptides together with self-MHC molecule.

In some organ and tissue graft (heart, kidney, pancreas, etc.), the APC's of the donor graft travel to the host regional lymph nodes. These APC's are dendritic cells known as passenger leukocytes (Fig. 15.5), which express high degree of MHC II molecules and normal MHC I molecule. These passenger leukocytes, because of their allogeneic MHC molecules are recognized as foreign and therefore, stimulate activation of T lymphocytes in the lymph node of the host.

Not always, the passenger cells play the role of antigen processing. Skin graft, particularly, Langerhans' cells and endothelial cells, which express both MHC I and MHC II are involved in antigen processing and immune stimulation.

The degree and type of immunological response depends on the type of transplant. Even if there is tissue incompatibility, there are certain privileged sites (such as the eye and brain), where transplantation does not evoke immune reactions. In others such as kidney, heart, pancreas, etc. effector lymphocytes are generated in the regional lymph nodes and are carried back by the lymphatics to mount immunological attack on the graft. Recognition of allogens, present on the transplant, induces marked degree of T cell proliferation in the host and the majorities are $CD4^+$ cells. These cells recognize class II alloantigens directly or alloantigen-peptide complex, presented by host APCs. The increased population of $T_h$ cells is thought to play a key role in inducing various effector mechanism of allograft rejection.

### *Effector Stage*

A number of mechanisms play a role in allograft rejection. Among all, the CMI is most common. In CMI, both delayed hypersensitivity ($T_h1$ mediated) reaction and cytotoxic T lymphocyte (CTL)-mediated reaction participated in allograft rejection. Less common are antibody plus complement-mediated ly-

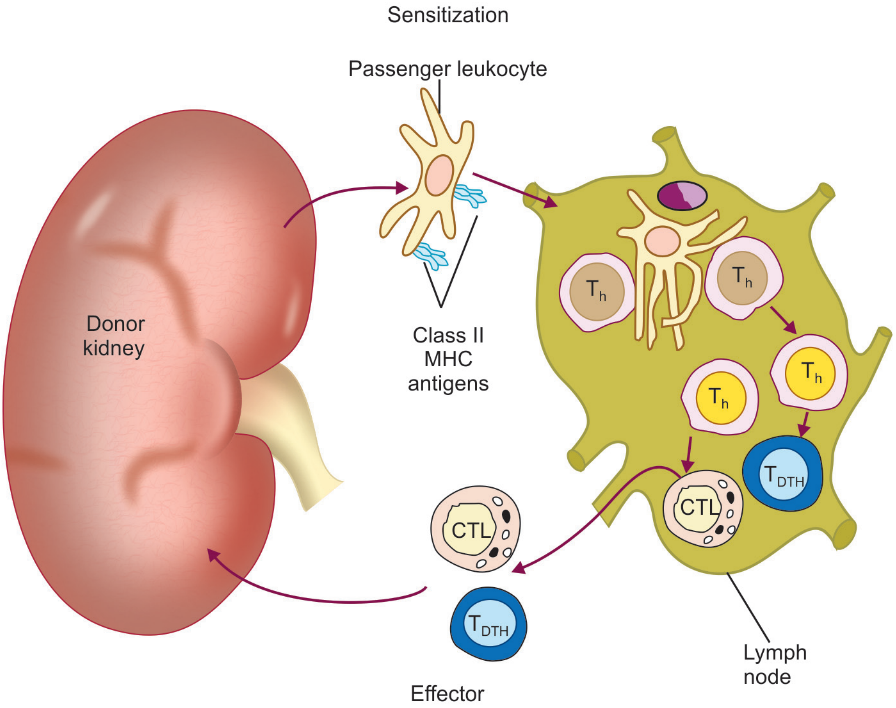


**Fig. 15.5:** Mechanism of graft rejection. Migration of passenger leukocytes from a donor graft to regional lymph nodes of the recipient, results in the activation of the $T_h$ cells in response to different class II MHC antigens expressed by the passenger leukocytes. These activated $T_h$ cells then induce generation of $T_{DTH}$ cells and/or CTLs, both of which mediate graft rejection.

sis and also antibody-dependant cell-mediated cytotoxicity (ADCC). The distinguishing characteristic feature of graft rejection in CMI is influx of T cells and macrophages into the graft. The infiltration of macrophage to the site, is mostly due to cytokines produced by T helper (CD4) cells. Recognition of foreign MHC I alloantigens by $CD8^+$ cells lead to CTL-mediated killing of cells.

T helper cells play a central role through their cytokines such as interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-beta (TNF-β). IL-2 promotes T cell proliferation and also help in generation of effector CTLs. IFN-γ is the main cytokine, which recruits macrophages to the graft and their subsequent activation into more destructive cells. TNF-β has got direct cytotoxic effect on graft cells. A number of cytokines (IFN-α, IFN-β, IFN-γ, TNF-α and TNF-β) increase class I and class II MHC expression on the allogeneic cells and APC's. During rejection episode, the level of cytokines increased inducing variety of cells to the graft. As the allograft rejection begins, localized production of IFN-α induces MHC II expression on endothelial cells and myocytes and making these cells target for CTLs (Fig. 15.6).

## Tempo of Rejection

Graft rejection reactions have various time courses and depend upon the type of tissue

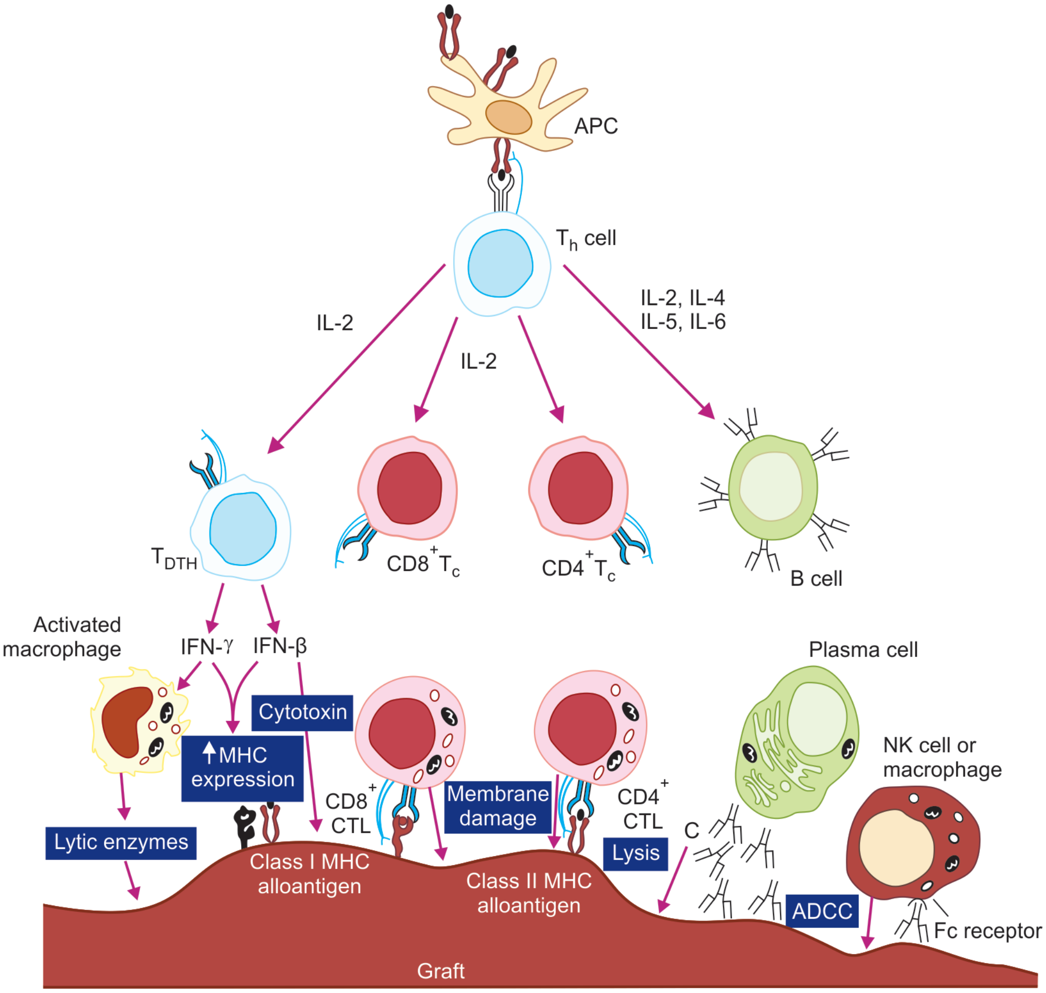

APC
Th cell
IL-2
IL-2
IL-2, IL-4
IL-5, IL-6
TDTH
CD8+Tc
CD4+Tc
B cell
Activated macrophage
IFN-γ
IFN-β
Plasma cell
Cytotoxin
↑MHC expression
CD8+ CTL
Membrane damage
CD4+ CTL
NK cell or macrophage
Lytic enzymes
Lysis
C
Class I MHC alloantigen
Class II MHC alloantigen
ADCC
Fc receptor
Graft

**Fig. 15.6:** Effector mechanisms involved in allograft rejection. The generation or activity of various effector cells depends directly or indirectly on cytokines secreted by activated $T_h$ cells (ADCC, antibody-dependent cell-mediated cytotoxicity; C, complement).

or organ graft and the effector mechanisms involved the following rejection.

### *Hyperacute Rejection*

Hyperacute rejection occurs within 24 hours of the graft implantation in patients those who have autoantibodies against the graft. Anti-HLA antibodies are induced by prior blood transfusion, multiple pregnancies or the rejection of the previous graft. Antigen-antibody complexes that activate complement system, causing an increase rush of neutrophils to the graft. The ensuing inflammatory reaction causes massive blood clot within the capillaries, preventing vascularization of the graft (Fig. 15.7) and block the microvasculature depriving the graft of blood supply.

### *Acute Rejection*

Usually begins in the first few weeks after the transplantation. The acute rejection is due to the primary activation of T cells and the consequent triggering of various effector mechanisms. If the patient had the history of reaction earlier (i.e. already sensitized), the secondary reaction of T cells occur leading to an accelerated cell-mediated rejection response.

### *Chronic Rejection*

Reaction develop months or years after transplantation. The rejection is due to combination of CMI response and antibody-mediated immune response. The walls of the blood vessels in the graft thicken and eventually become blocked. Chronic rejection may be due to several causes, such as a low-grade cell-mediated rejection or the deposition of antigen and antibody in the graft tissue. Grafts may also be damaged by the recurrence of the disease process for which the transplantation was needed.

### *Graft-versus-host Disease*

A number of malignant and non-malignant hematologic diseases, including leukemia,

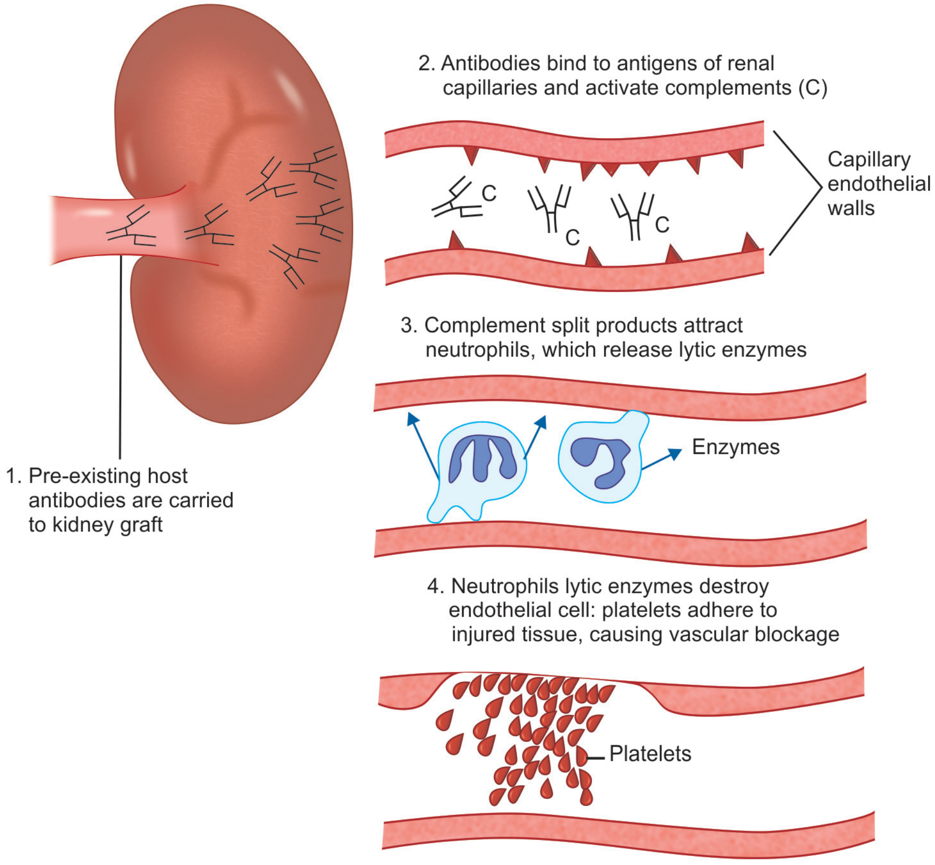

2. Antibodies bind to antigens of renal capillaries and activate complements (C)
C
C
C
Capillary endothelial walls
3. Complement split products attract neutrophils, which release lytic enzymes
Enzymes
1. Pre-existing host antibodies are carried to kidney graft
4. Neutrophils lytic enzymes destroy endothelial cell: platelets adhere to injured tissue, causing vascular blockage
Platelets

**Fig. 15.7:** Steps in the hyperacute rejection of kidney graft. In this type of rejection, the graft never becomes vascularized.

lymphoma, aplastic anemia, thalassemia, major and minor and many immunodeficiency states are being treated by bone marrow transplantation. In the usual procedure, the recipient of the bone marrow transplant is immunologically suppressed, preventing the rejection of transplant. However, the immunocompetent cells in the transplanted bone marrow may recognize histocompatibility antigens in the recipient cells, as foreign and attack the host tissue. This is a graft-versus-host (GVH) response and the resulting damage is graft-versus-host disease (GVHD). The most immediate and serious threat comes from the mature T cells, because they are capable of generating rapid and severe GVHD responses. The activation and proliferation of these T cells and subsequent production of cytokines generate inflammatory reactions in skin, gastrointestinal (GI) tract and liver. Generalized erythroderma of skin, GI hemorrhage and liver failure follow. This response is prevented or minimized by giving the transplant recipient, a regime of immunosuppressive drugs. In another approach, the donor marrow is treated with either anti-T cell sera or mAbs specific for T cell, there by destroying the reactive T cells.

The major clinical features of GVH reaction in animals are retarded growth, emaciation, diarrhea, hepatosplenomegaly, lymphoid atrophy and anemia terminating fatally. The syndrome has been called runt disease.

## STUDY QUESTIONS

### Essay Questions

1. Define transplantation. Write about the sequence of events occurring in allograft rejection.
2. Classify grafts. Explain graft-versus-host reaction.

### Short Notes

1. Allograft.
2. Xenograft.
3. Graft-versus-host reaction.
4. HLA typing.

## SUGGESTED READING

1. Black JG. Microbiology. Principles and Applications, 3rd edition. 1996.
2. Borish L. Genetics of allergy and asthma. Ann Allergy Asthma Immunol. 1999 (May) 82(5): 413-24; quiz 424-26.
3. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
4. Goldsby RA, Kuby TJ. Immunology, 6th edition. 2007.
5. Greenwood D, Slack R. Medical Microbiology, 15th edition. 1997.
6. Jawetz. Textbook of Microbiology, 25th edition. 2010.
7. Lippincott.Immunology.1stIndianprint;2008.
8. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. 2006.
9. Stites. Basic and Clinical Immunology, 8th edition. 2007.

# 16

# Immunohematology

## DISCOVERY OF BLOOD GROUP

Blood transfusion had been attempted from very early times, but such attempts were fruitless and often fraught with disastrous consequences. Blood transfusion became scientifically feasible only after the discovery of blood groups by Landsteiner.

The ABO system is the most important of all the blood group systems and its discovery made blood transfusion possible. Cells, which failed to agglutinate with any of the serum samples were designated group ‘O’, while cells agglutinating in the two different patterns were called A and B respectively. The fourth group AB was described later by his pupils von Decastello and Sturli (1902). In 1930, Landsteiner was awarded Nobel Prize for his discovery of human blood groups. No other blood group antigens were discovered for the next 25 years. Using rabbit antisera to different samples of human red cells, Landsteiner and Levine (1926) discovered the MN and P antigens. Landsteiner and Wiener (1940) raised rabbit and guinea pig antisera against rhesus (Rh) monkey erythrocytes and tested them against human red cells. This led to the discovery of the Rh factor.

Many more blood group antigens have been identified subsequently, mostly by studying antibodies in patients, who had received multiple blood transfusions or mother of infants with hemolytic disease. The main blood group systems with the year of their discovery are shown in Table 16.1.

**Table 16.1** Blood group systems with the year of their discovery

| Blood groups | Years | Blood groups | Years |
|---|---|---|---|
| ABO | 1900 | Duffy | 1950 |
| MN | 1926 | Kidd | 1951 |
| P | 1926 | Diego | 1955 |
| Rh | 1940 | Yt | 1956 |
| Lutheran | 1945 | Kg | 1962 |
| Lewis | 1946 | Dombrock | 1965 |
| Kell | 1946 | Colton | 1967 |

### ABO Blood Group System

The ABO system contains four blood groups and is determined by the presence or absence of two distinct erythrocytes. Red cells of group A carry antigen A, cells of group B carry antigen B and cells of group O have neither A or B antigens. The four groups are also distinguished by the presence or absence of two distinct isoantibodies in serum. The serum contains the isoantibodies specific for antigen that is absent on the red cell. The serum of a group A individual has anti-B antibody, group B has anti-A and group O has both anti-A and anti-B, while in group AB both anti-A and anti-B are absent (Table 16.2).

Group A is subdivided into A1 and A2. Antiserum of group A agglutinates group A1 cells powerfully, but A2 cells agglutinates weakly. About 80 percent of group A blood is A1 and 20 percent is A2. Other A subgroups (A3, A4, A5) have also been described, but they are not clinically relevant.

Blood group antigens are inherited according to simple Mendelian laws. Their synthesis is determined by allelomorphic genes A, B and O. Genes A and B give rise to the corresponding antigens, but O is an amorphous and does not produce an antigen. The frequency of ABO distribution differs in different people. Group 'O' is the commonest group and AB is rarest.

In India, distribution of ABO blood groups is approximately O—40%; A—22%; B—33% and AB—5%. Anti-A and Anti-B isoantibodies, appear in the serum of infants, by about the age of 6 months and persists thereafter. These are called natural antibodies, because they seem to arise under genetic control without any apparent antigenic stimulation. Immune isoantibodies may develop following ABO incompatible pregnancy or transfusion.

'H' Antigen: Red cells of all ABO groups possess a common antigen, the 'H' antigen of H substance, which is a precursor for the formation of A and B antigens. Due to its universal distribution, the 'H' antigen is not ordinarily important in grouping or blood transfusion. Bhende et al (1952) from Bombay reported a very rare blood group known as the Bombay OH blood group, such individuals will have anti-A, anti-B and anti-H antibodies (absence of A, B and H antigens). So that their serum will be incompatible with all red cells except of those with the same rare blood group.

The blood group antigens are glycoprotein and are found in almost all the tissues and body fluids. They are found in secretions (saliva, gastric juice, sweat) of only about 75 percent of all persons. Such persons are called 'secretors' and those who lack these antigens in secretion are called 'non-secretors'.

Substances specifically agglutinating A or B antigens have been detected in some plants (e.g. a potent anti-A, agglutinin has been extracted from Dolichos biflorus). Blood group agglutinins of plant origin are known as lectins.

## Rh Blood Group

Levine and Stetson (1939) demonstrated a new type of antibody in the serum of a woman who developed severe reactions following transfusion of her husband's ABO compatible blood. She had recently delivered a stillborn infant with hemolytic disease. They suggested that the woman may have been sensitized by some antigen inherited by the fetus from its father.

**Table 16.2** Distribution of ABO antigens and antibodies in red cells and serum

| Red Cells | | | Serum | |
|---|---|---|---|---|
| Group | Antigen present | Agglutinate by serum of group | Antibody present | Agglutinate cells of group |
| A | A | B, O | Anti-B | B, AB |
| B | B | A, O | Anti-A | A, AB |
| AB | A and B | A, B, O | None | None |
| O | None | None | Anti-A and anti-B | A, B, AB |

Landsteiner and Wiener (1940), identified in the red cells of the majority of persons tested, an antigen that reacted with rabbit antiserum to Rh monkey erythrocytes. This antigen was called the Rh factor. The new type of antibody described by Levine and Stetson was identified as anti-Rh factor antibody. Wiener and Peter (1940) demonstrated anti-Rh antibody in some persons who had received ABO compatible transfusion. Levine and his colleagues (1941) proved that Rh sensitization was the cause of hemolytic disease of the newborn.

Fisher postulated that Rh antigens are determined by three pairs closely linked allelomorphic genes, *Cc, Dd* and *Ee*. Every individual possesses one member of each pair of these genes derived from each parent.

The designations employed by the two systems for the different Rh type are as follows: For routine purpose, the typing of persons as Rh positive or Rh negative depends on the presence or absence of antigens D (Rho) on red cells and hence accomplished by testing with anti-D (anti-Rh) serum. This is because D is the most powerful Rh antigen and accounts for the vast majority of Rh incompatibility reaction (Table 16.3). Among Indians, approximately 93 percent are Rh positive and 7 percent are Rh negative.

There are no natural anti-Rh antibodies in the serum. They arise only as a result of Rh incompatible pregnancy or transfusion.

## APPLICATIONS OF BLOOD GROUPS

### Blood Transfusions

Indications: Transfusion of blood and blood components has been helpful in long-term survival of patients with various types of illness:

- Thalassemia major
- Hematological malignancies
- Sickle cell crisis (trauma, acute hemorrhage during surgery).

The transfusions of blood and its role in saving the patients life cannot be over-emphasized. But blood also has its own adverse effects. It is a double-edged sword and must be administered only when the benefits over weigh the risks. The hazards are:

1. Transfusion transmitted diseases.
2. Hemolytic transfusion reactions.

#### *Transfusion Transmitted Diseases*

Iatrogenic diseases are transmitted through blood transfusion. The blood may contain bacteria, virus, parasite or neoplastic cells, which may inadvertently, negligently, accidentally or through faulty screening technique and oversight may gain entrance. Endotoxins of gram-negative bacteria, which may gain entrance through vein puncture. Contaminated needle, collecting set and blood collected from donor suffering from mild bacteremia, leads to endotoxic shock and disseminated intravascular coagulation (DIC).

Viruses like hepatitis B, human immunodeficiency virus (HIV) and cytomegalovirus (CMV) can be transmitted through blood transfusion. Parasites like malaria parasite and spirochete such as *Treponema pallidum* can be transmitted. All the above organisms should be screened in donor's blood before transfusion. Malignant cells also should be screened for in peripheral smear.

**Table 16.3** Fisher and Wiener postulated blood group systems

| Rh type | Fisher | Wiener |
|---|---|---|
| Rh positive | CDe<br>cDE<br>cDe<br>CDE | Rh1<br>Rh2<br>Rho<br>Rhz |
| Rh negative | Cde<br>cdE<br>cde<br>CdE | Rh$^{1}$<br>Rh$^{11}$<br>Rh<br>Rhy |

### *Hemolytic Transfusion Reactions*

Hemolytic transfusion reactions occur after the transfusion of incompatible blood, the common example being transfusion of 'A' group to a B group individual.

In patients with mismatched transfusion, there will be fever, chill, facial flushing, chest pain, hypotension, nausea, vomiting, hemoglobinuria, anuria, oliguria and shock.

The antibodies, which are formed to 'ABO' system of antigens are immunoglobulins (IgM) in nature. So following transfusion of mismatched blood, there is agglutination, complement activation and intravascular hemolysis (Table 16.2).

Delayed hemolytic transfusion reactions, generally occur in individuals who have received repeated transfusion of 'ABO' compatible blood, but that is incompatible for other blood groups (Rh, Kidd, Kell and Duffy) antigens. These antigens induce IgG, instead of IgM as against 'ABO' group antigen, which have weak agglutinating and complement-activating ability.

Hyperacute graft rejection is related to transfusion reactions. This is because the 'ABO' group antigens are also expressed on kidney cells. Hyperacute graft rejection occurs when a graft recipient has preformed antibody against the grafted tissue.

### *Hemolytic Disease of the Newborn*

Hemolytic disease of the newborn (HDN) occurs when the mother has been sensitized to antigen on the infant's erythrocytes and makes IgG antibodies to these antigens. These antibodies cross the placenta and react with the fetal erythrocytes, causing their destruction (Figs 16.1A to D).

Erythrocytes from $Rh^+$ ($RhD^+$) fetus leak into the maternal circulation usually during birth. If mother is $Rh^-$ this stimulates production of anti-Rh antibody of the IgG class. Sensitization of $Rh^-$ mother to Rh-positive erythrocytes usually occurs during birth of the first $Rh^+$ infant. Thus, the first incompatible infant usually unaffected, whereas in subsequent pregnancy, these IgG antibodies cross the placental barrier and attack the fetal red cells causing HDN. An increasing risk of subsequent infants being affected, as the mother is desensitized with each successive pregnancy.

The risk of HDN due to Rh incompatibility is negligible, if the father is of different blood group to the mother. The $Rh^-$ mothers destroy the $Rh^+$ erythrocytes more rapidly, as they are Rh incompatible as well as 'ABO' incompatible. The $Rh^+$ erythrocytes are so quickly destroyed by ABO incompatibility that they would not be available to sensitize the maternal immune system to Rh 'D' antigen.

Direct and indirect Coombs' tests are used to detect anti-Rh antibodies in the newborn and mother, respectively.

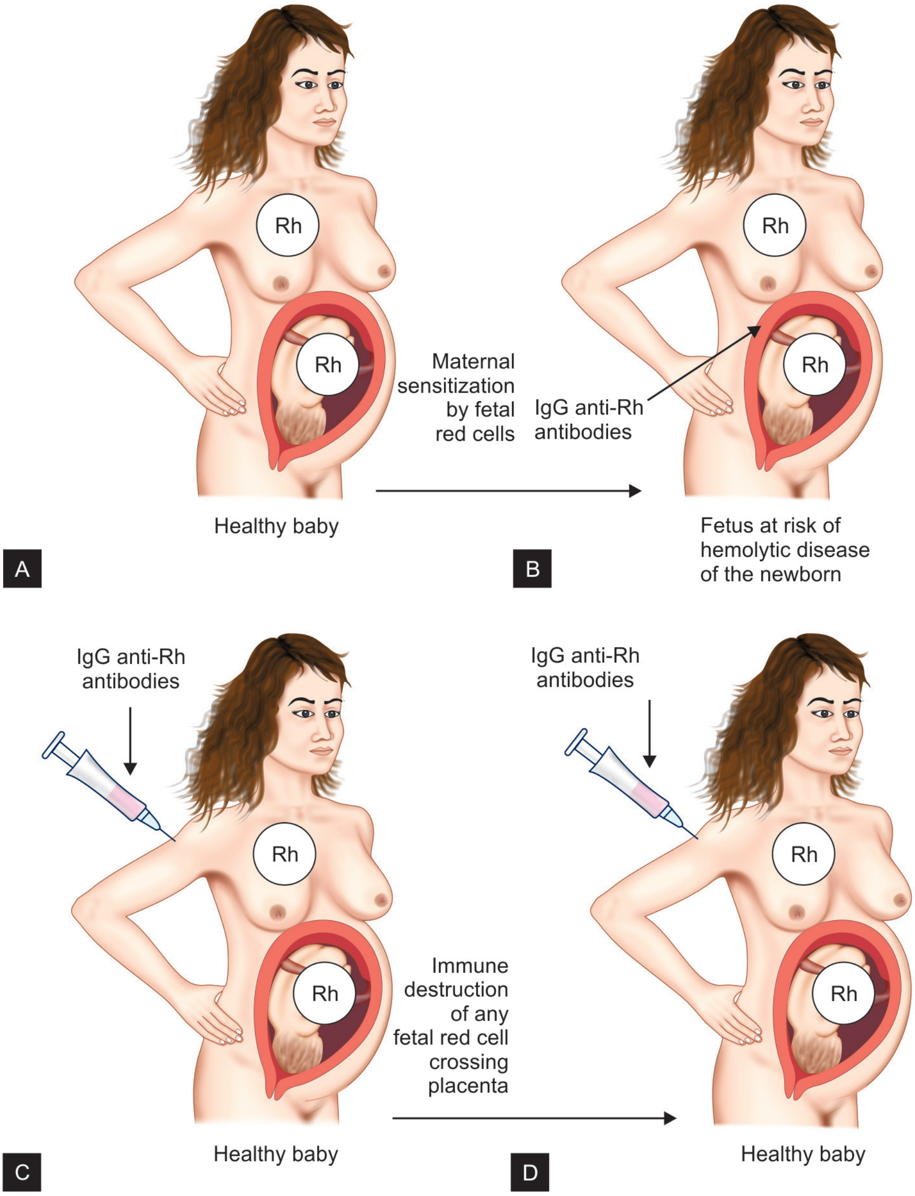
## AUTOIMMUNE HEMOLYTIC ANEMIA

Autoimmune hemolytic anemia (AIHA) is a condition, in which the patients produce antibodies to their own erythrocytes (blood group antigens) spontaneously. In this condition, direct antiglobulin test (Coombs' test) becomes positive. These antibodies are either directed towards erythrocytic antigens or immune complex adsorbed on to the erythrocytes surface.

The autoimmune hemolytic anemia can be divided into three types, depending upon the nature of antibodies produced against erythrocytic antigens.

### Warm-reactive Autoantibodies

Autoantibodies react with antigen at 37°C and are formed to Rh system antigens


Rh
Rh
Maternal sensitization by fetal red cells
Healthy baby
A
IgG anti-Rh antibodies
Rh
Rh
Fetus at risk of hemolytic disease of the newborn
B
IgG anti-Rh antibodies
Rh
Rh
Immune destruction of any fetal red cell crossing placenta
Healthy baby
C
IgG anti-Rh antibodies
Rh
Rh
Healthy baby
D

**Figs 16.1A to D**: Immunoprevention of hemolytic disease of newborn. **A.** In the first pregnancy of Rh-negative mother, the baby is normal, but Rh positive and at some point (e.g. during delivery) fetal red blood cells (RBCs) cross into the maternal circulation, giving rise to production of IgG anti-Rh antibodies; **B.** During subsequent pregnancies, the IgG antibodies cross the placenta and cause fetal anemia and heart failure; **C.** Prevention relies on removing the red cells, as they cross the placenta by administering anti-Rh antibody to the mother, e.g. at delivery; **D.** This prevents sensitization, but must be given in all subsequent pregnancies as well.

(determinants of RhC, RhE and RhD). They differ from the antibodies responsible for transfusion reactions, in that they appear to react with different epitopes. Most of the hemolytic anemias are of unknown etiology, but some are associated with other autoimmune diseases. The anemia may be attributed to the accelerated clearance of sensitized erythrocytes by spleen or by complement-mediated lysis.

### Cold-reactive Autoantibodies

Autoantibodies react with antigens at below 37°C. The antibodies are primarily IgM in nature and fix complement strongly. They are specific for Ii blood group system. The I and i epitopes are expressed on the precursor polysaccharides that produce the ABO system epitopes and are the result of incomplete glycosylation of the core polysaccharide. Most cold reactive autohemolytic anemia occurs in old people. The cause is not known. In some individuals, it follows *Mycoplasma pneumoniae* infection. It is thought that the antibody produce towards *M. pneumoniae* cross-reacts with erythrocytes and cause hemolysis.

### Drug-induced Reaction Against Blood Components

Drugs bind to the blood cells (RBCs, platelets, etc.) and the antibodies are produced against drug-bound cells. There is complement activation, which is followed by cell lysis. For example, the drug, sedormid, causes destruction of platelets leading to sedormid purpura. Similar mechanism holds responsible for causation of hemolytic anemia, while treating patients with quinine, penicillin and sulfonamides.

Drug-antibody immune complex may be deposited on the red cells via C3b receptor (CR1). Damage ensues via complement-mediated lysis.

Drugs/metabolites, such as methyldopa may adsorb on red cells and bring about a change in erythrocyte cell surface antigen. Thus, there is breakdown of cell tolerance. Destruction of erythrocytes follows complement-mediated lysis.

### Autoantibodies to Antineutrophil Cytoplasmic Antigens

Antineutrophil cytoplasmic antibodies (ANCAs) are associated with a number of diseases. In systemic lupus erythematosus (SLE), there is formation of antibodies to myeloperoxidase (MPO). The antibodies are located in perinuclear granules (p-ANCA). Other granule components may also act as antigen in SLE. Antibodies to protein-3, a cytoplasmic antigen (c-ANCA) is also associated with Wegener granulomatosis.

### Autoantibodies to Platelets

Autoantibodies to platelets are formed in about 70 percent of cases of idiopathic thrombocytopenic purpura (ITP). Platelet deficiency occurs due to accelerated removal of platelets from circulation, primarily by splenic macrophages.

Often, ITP follows bacterial and viral infections. At time ITP is associated with other autoimmune diseases including SLE.

## BLOOD GROUP AND DISEASES

It has been reported that the blood group antigens, in some cases, are affected in certain diseases. The acquisition of B antigens in group A person has been reported. In leukemia, the blood group antigens become weak.

Bacterial contamination *(Pseudomonas aeruginosa)* of red cells suspension unmasks the hidden antigen (T antigen), normally, present in all human erythrocytes. As anti-T agglutinins are normally present in all human sera, the red cell suspension becomes agglutinable by all blood group sera. This is known as Thomsen-Friedenreich phenomenon. Such panagglutinability of red cells has, occasionally been seen in persons suffering from systemic bacterial infections.

The correlation of blood group antigens with the susceptibility of certain diseases have been reported. Association of blood group 'O' with peptic ulcer and group 'A' with cancer of the stomach is known long since.

## STUDY QUESTIONS

### Essay Questions

1. What adverse reactions occur following blood transfusion?
2. Explain how the Rh incompatibility manifests in newborn and how this disease might be prevented.
3. Briefly describe the mechanism of autoimmune hemolytic anemia.

### Short Notes

1. Name the infections transmitted through blood.
2. Blood group typing and cross-matching.
3. Autoantibodies to platelets.

## SUGGESTED READING

1. Black JG. Microbiology: Principles and Applications, 3rd edition. USA: Printice Hall College Div; 1996.
2. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
3. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
4. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
5. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. Lange Basic Science, USA: McGraw Hill; 2010.
6. Male David, Brostoff Jonathan, Roth David B, et al. Immunology, 7th edition. Mosby Elsevier; 2006.
7. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
8. Tortora Gerard J, Funke, Berdell R, Case Christine L. Microbiology: an Introduction, 10th edition. USA; 1998.

# Immunoprophylaxis Vaccines

# 17

The purpose of immunization in one individual is to prevent diseases. The objective of immunization program has been responsible for spectacular advances in combating the infectious diseases such as smallpox, poliomyelitis, tetanus, diphtheria, etc. Since 1977 smallpox has been eradicated, equally encouraging is the predicted eradication of polio.

## HISTORICAL OVERVIEW

The discipline of immunology had its roots in the early vaccination trials of Edward Jenner and Louis Pasteur. Edward Jenner's introduction of vaccinations with cowpox virus (1796) to protect against smallpox was the first documented use of a live, attenuated viral vaccine and the beginning of modern immunization. Robert Koch was the pioneer to demonstrate the specific bacterial cause of anthrax (1876). Subsequently, the causes of several common illnesses were rapidly identified.

The control of number of the diseases that cause significant mortality has made outstanding progress, but there remains an urgent need for vaccines against others. Every year, millions are dying from malaria, tuberculosis (TB) and acquired immune deficiency syndrome (AIDS) for which there are no effective vaccines.

In addition to the challenges presented by diseases for which there are no effective vaccines, there remains the need to improve the available vaccines as regard to safety and efficacy and cost-effectiveness, especially in developing country. The World Health Organization (WHO) estimates that 20% to 35% of infant deaths, in the world, are due to diseases that could be prevented by existing vaccines. Some potential vaccines have unacceptable side effects and others' worsen the disease, especially for the immunodeficient subjects. Therefore, stringent testing is an absolute necessity, as the vaccines will be given to healthy subjects.

## ACTIVE AND PASSIVE IMMUNIZATION

Immunity against infections can be achieved by active and passive immunization. In active immunization, the immunity is achieved naturally by suffering, clinically or subclinically from the disease or by artificial means, such as vaccines (Table 17.1). Similarly, in passive immunization, the immunity is obtained naturally from mother to fetus by transfer of antibody (from mother to infant via milk or artificial injection of preformed antibody) (Table 17.2).

### Passive Immunization

Jenner and Pasteur are the stalwarts who pioneered vaccines. So, also Emil von Behring and Hidesaburo Kitasato are well recognized by their contributions to passive immunity. In fact these investigations, for the first time, showed that the immunity could be transferred passing from one individual to other by serum. Common agents used for passive immunization are mentioned in Table 17.2.

## Active Immunization

The goal of the active immunization is to elicit protective immunity and immunological memory. When active immunization is successful, a subsequent exposure produce heightened response, leading to the elimination of pathogen or prevention of the disease mediated by its products. Active immunization can be achieved by natural infection with the microorganism or it can be acquired artificially by administration of vaccines. In active immunization, as the name implies, the immune system plays active role by producing antigen-reactive T or B cells. Some of them remain as memory cells. The recommended program of childhood immunization in India is outlined in Table 17.1.

## Routine Immunization

While introducing immunization schedule, certain factors are taken into consideration. The schedule is based on the prevalence of the infectious diseases, their public health importance, availability of suitable vaccines, the cost-benefit factors and the logistics. In India, the Expanded Program on Immunization (EPI) and the Universal Immunization Program (UIP) has been able to afford protection against vaccine-preventable diseases (VPDs). The National Immunization Schedule, in force, in India shown in Table 17.1.

**Table 17.1** National Immunization Schedule (India)

| Age | Vaccine |
|---|---|
| At birth | Bacille Calmette-Guérin (BCG), oral polio vaccine-0 (OPV-0) |
| 6 week | BCG-2, diphtheria, pertussis, tetanus, (DPT-1), OPV-1 |
| 10 week | DPT-2, OPV-2 |
| 14 week | DPT-3, OPV-3 |
| 9 month | Measles |
| 16–24 month | DPT, OPV |
| 5–6 year | Diphtheria-3 (DT-3) |
| 10 year | Tetanus toxoid-4 (TT-4) |
| 16 year | TT-4 |
| For pregnant women | TT-1 or buster |
| 1 month after TT-1 | TT-2 |

Note:

1. For institutional birth only. OPV-0 is additional and not to be counted for the primary course of third doses starting at 6 week.
2. Only for infant not given BCG at birth.
3. A second dose of DT to be given to children with no documentary evidence or history of primary DPT immunization.
4. A second dose of TT to be given after month to those with no record or history of prior DPT, DT or TT immunization.
5. For prevention of tetanus in the neonate primarily, but also in the mother.

## DESIGNING VACCINES OF ACTIVE IMMUNIZATION

The designer must keep in mind certain factors for developing successful vaccine. The first requirement is the development of appropriate immune response in order to deal with the organism, what is often critical that, which branch of the immune system antibody -mediated immunity (AMI) or cell-mediated immunity (CMI) is to be activated, depending on the structural contents and the nature of the microorganisms. A second factor is the development of immunological memory. If a vaccine induces only protective primary response and fails to induce memory cells, the host is not protected on subsequent infection by the same microorganisms. The role of memory cells also, is dependent on the incubation period of the pathogen. For example, in the case of influenza virus, which has a short incubation period (1 or 2 day). The disease symptoms are already on the way before the memory cells are activated. Effective protection against influenza can be achieved by repeated immunization to maintain a high level of neutralizing antibody. For pathogen, with long incubation period (poliovirus), requires more than 3 days to begin to infect central nervous system (CNS). An incubation period of this length gives the memory B cells more time to respond by producing high level of serum antibody.

In addition to the factors already mentioned, the effective protection against the intended pathogen must occur without danger of causing disease or producing severe side effects. Besides, the vaccine must be economically feasible for production and be suitably stable for storage, transport and use.

### Whole Organism Vaccines

Many of the common vaccines, which are in use fall into inactivated (killed) or live, but attenuated (avirulent) bacterial cells or viral particles (refer Table 17.2).

**Table 17.2** Classification of common vaccines for humans

| **Disease or pathogen whole organisms** | |
|---|---|
| **Bacterial cells** | **Types of vaccine** |
| Anthrax | Inactivated |
| Cholera | Inactivated |
| Pertussis* | Inactivated |
| Plague | Inactivated |
| Tuberculosis | Live attenuated BCG† |
| Typhoid | Live attenuated |
| **Viral particles** | |
| Hepatitis A | Inactivated |
| Influenza | Inactivated |
| Measles | Live attenuated |
| Polio (Sabin) | Live attenuated |
| Polio (Salk) | Inactivated |
| Rabies | Inactivated |
| Rotavirus | Live attenuated |
| Rubella | Live attenuated |
| Varicella zoster (chickenpox) | Live attenuated |
| Yellow fever | Live attenuated |
| **Whole organisms purified macromolecules** | |
| *Toxoids* | |
| Diphtheria | Inactivated exotoxin |
| Tetanus | Inactivated exotoxin |
| **Capsular polysaccharides** | |
| *Haemophilus influenzae* type b | Polysaccharide protein carrier |
| *Neisseria meningitidis* | Polysaccharide |
| *Streptococcus pneumoniae* | 23 distinct capsular polysaccharides |
| Surface antigen | Recombinant surface antigen |

*There is also acellular pertussis vaccine.

†Bacille Calmette-Guérin (BCG) is an avirulent strain of *Mycobacterium bovis*.

### *Attenuated Bacterial or Viral Vaccines*

In some cases, microorganisms can be attenuated, so that the pathogenicity is destroyed, but antigenic property is retained. Attenuation can be achieved by growing a pathogenic bacteria or virus for a prolonged periods under abnormal culture conditions. This procedure selects mutants, which are capable of growing in abnormal culture conditions and therefore, less capable of growth in natural host. The common example is the BCG vaccine, which is the attenuated strain of *Mycobacterium bovis*. This vaccine was prepared by growing *M. tuberculosis* on a medium containing increasing concentration of bile. Several examples, such as Sabin polio, measles and other vaccines have been successfully attenuated.

*Advantages:* The attenuated vaccines have advantages and disadvantages. Because of their capacity for transient growth, such vaccines provide prolonged immune system exposure to the epitopes of the attenuated vaccine, so that there is increased immunogenicity and production of memory cells. Therefore, booster doses may not be required. This property of the vaccine is more relevant in developing country like India, where majority do not turn up for the second and subsequent doses of the vaccines. The other advantage of the live attenuated vaccine is the production of CMI local immunity. The attenuated Sabin vaccine colonizes the intestine and enables to induce production of secretory immunoglobulin A (IgA), which serves an important defense against naturally acquired poliovirus. The vaccine induces both IgM and IgG class.

*Disadvantages:* One of the important disadvantage of the attenuated strain is the possibility of their reversion to virulent form. The rate of reversion of the Sabin polio vaccine (OPV) is one case in 4 million doses of vaccine. Another danger with the attenuated vaccine is the presence of other virus as contaminants [simian virus-40 (SV40)], oncogenic virus in monkey kidney cultures, used for Sabin vaccine production). Attenuated vaccines may rarely, be associated with complications similar to those seen in natural diseases. There may be also vaccine-mediated immune suppression, as that happens in measles vaccine (Edmonston-Zagreb strain).

Presently, genetic engineering techniques play important role to attenuate viruses irreversibly by selectively removing the genes that are necessary for virulence (herpes virus). More recently, a vaccine for rotavirus has been developed using genetic engineering techniques to modify an animal rotavirus to contain antigens present in human rotavirus.

### *Killed Bacterial or Viral Vaccines*

Another approach to prepare vaccine is to inactivate the bacteria by heat or chemicals. Heat inactivation may be unsatisfactory due to extensive denaturation of protein. Chemical inactivation with formaldehyde or various alkylating agents has been successful. Salk polio vaccine and the pertussis (whooping cough) vaccines are the examples of chemically (formaldehyde)-inactivated vaccines.

The inactivated vaccines, unlike live attenuated vaccines, are given in repeated booster doses, because, the organisms are killed and do not multiply in the tissue. They are usually given intramuscularly and they produce predominantly humoral immunity. If inactivation is improper this may lead to serious untowards complications. A serious complication (paralytic polio) with first Salk vaccines arose, when formaldehyde failed to kill all the viruses. Pertussis vaccine may also cause encephalitis, if not inactivated properly. Therefore, presently acellular pertussis vaccine (Table 17.2) has replaced the whole organism vaccines.

## Purified Macromolecules as Vaccines

Three types of vaccines are currently available. They are capsular polysaccharides, inactivated exotoxins and recombinant surface antigens (refer Table 17.2).

### *Polysaccharide Vaccine*

The virulence of the capsulated bacteria depends on the presence of capsules, which prevent phagocytosis. But if the capsulated bacteria are coated with antibodies and/or complement (opsonin), they can be easily phagocytosed by macrophages and neutrophils. This is the principle of purified polysaccharide vaccines.

Presently, polysaccharide vaccines are available for pneumococcus, *Neisseria meningitidis* and *Haemophilus influenzae*. Vaccine for pneumococcus, which causes pneumococcal pneumonia consists of 23 antigenically capsular polysaccharides. The vaccine induces opsonizing antibodies. One limitation of polysaccharide capsular antigen is that they cannot activate T helper cells ($T_h$cell). They activate B cells in thymus-independent type-2 manner, resulting in IgM production. There is no class switching and development of the memory cells. One way to involve the $T_h$cells directly in the response to a polysaccharide antigen, is to conjugate the antigen to some sort of protein carrier. For example, the vaccine for *H. influenzae* type b (Hib), which is a major cause of bacterial meningitis, in children, under 5 years of age, consists of type 'b' capsular polysaccharide covalently linked with a protein carrier tetanus toxoid (TT).

### *Toxoid Vaccines*

In some bacterial diseases, the pathogens are toxigenic rather than invasive. The common examples are *Corynebacterium diphtheriae* and *Clostridium tetani*. They produce powerful exotoxins, which cause lesions. Vaccine against diphtheria and tetanus can be made by purifying the bacterial exotoxin and inactivating the toxins with formaldehyde to form toxoids. The toxoid when given in vaccine, induces antitoxoid antibodies, which are also capable of neutralizing the toxin. One of the problems was difficulty in obtaining the exotoxins in large quantity. This impediment has been overcome by cloning the exotoxin genes and impressing them in easily grown host cells. In this way, large quantities of exotoxins can be produced, purified and subsequently inactivated.

### *Recombinant Antigen Vaccines*

The genes encoding any immunogenic protein can be cloned and be expressed in bacteria, yeasts and other mammalian cells by recombinant deoxyribonucleic acid (DNA) technology.

A number of genes from surface antigen of viruses, protozoa and other pathogens have been successfully cloned into bacterial cell, insects, yeasts and the expressed antigen may be used in development of vaccines.

The first such recombinant antigen vaccine, approved and used, is the hepatitis-B surface antigen, this was developed in yeast cells. The recombinant yeast cells are grown in large fermenters and hepatitis-B surface antigen (HBsAg) accumulates intracellularly. These yeast cells are then harvested, disrupted in high pressure, releasing the recombinant antigens. Subsequently, they are purified by biochemical methods.

### *Recombinant Vector Vaccines*

Genes that encode major antigens of virulent pathogens can be introduced to attenuated viruses and bacteria. The attenuated organisms can serve as a vector, replicating within the host and expressing the gene products. The organisms, which serve as vectors and replicate in the host cells include vaccinia virus, canarypox virus, attenuated poliovirus, adenovirus and attenuated strains of *Salmonella*. Vaccinia virus is the most suitable vector widely used. This large complex virus can carry several dozens of foreign genes without impairing its capacity to infect host cells and replicate. Genetically engineered vaccinia vector vaccines can be administered, simply by scratching the skin, causing localized infection in host cells. The procedure for producing a vaccinia vector that carries a foreign gene from pathogen is outlined in Figure 17.1.

## Deoxyribonucleic Acid Vaccine

Recently, plasmid DNA encoding antigenic proteins are directly injected into the muscle of the recipient. DNA is taken up by the muscle cells and the expressed protein produce desired humoral immunity or CMI. The DNA, inside the muscle cells, remains integrated with the host cell DNA or in an episomal form. In addition to muscle cells, the dendritic cells around the area, also take up the plasmid DNA and express viral antigen.

Deoxyribonucleic acid vaccine, presently, proves better because of certain distinct advantages over other vaccines. The encoded protein expression occurs, in its natural form, without denaturation or modification. DNA vaccines induce both humoral and CMI. The DNA vaccine caused prolonged expression of antigen, therefore, generating immunological memory cells. Refrigeration is not mandatory, which helps the cost-effectiveness.

An improved method, for administering these vaccines, is to coat microscopic gold beads with the plasmid DNA and then deliver the coated particles through the underlying muscle with an air *gun* (gene gun). This will allow rapid delivery of vaccines to large population without the requirement of large number of needles and syringes.

At present, there are animal and human trials under way with several DNA vaccines, including those for, malaria, AIDS, influenza and herpes virus. Cytokine-producing genes, such as interleukin-12 *(IL-12)* gene, can be combined with DNA vaccine to produce optimal immune response. The expression of IL-12 at the site of immunization will stimulate $T_h1$ type of immunity, induced by the vaccine.

Certain drawbacks of DNA vaccine do exist. First, only protein antigen can be encoded and secondly, inability to use the DNA vaccine to provide mucosal immunity as that happens in oral and nasal spray vaccine.

## Synthetic Peptide Vaccines

Although, initially, the use of synthetic peptide as vaccines appeared very promising, it had not progressed much, as expected. The reason being, they are not immunogenic as proteins and they are not effective in producing both humoral and CMI. Their immunogenicity can be enhanced by conjugating adjuvants, but there are some other impediments, which have prevented their use. To produce a desired humoral or CMI, an understanding of the nature of T cell and B cell epitopes are required. In most pathogens, though the amino acid sequence of many important antigens is known, their three-dimensional structure is unknown. An effective memory response for both humoral and CMI, the generation of population of memory $T_h$ cells is imperative. A successful vaccine must therefore include immunodominant T cell epitopes. Synthetic peptides that represent immunodominant T or B cell epitopes are being evaluated as vaccines for several diseases.

## Multivalent Subunit Vaccines

One of the limitations of synthetic peptides and recombinant vaccine is that they are weakly immunogenic. In addition, they produce only humoral immunity, but not CMI. The most ideal synthetic peptide vaccine should contain both immunodominant B and

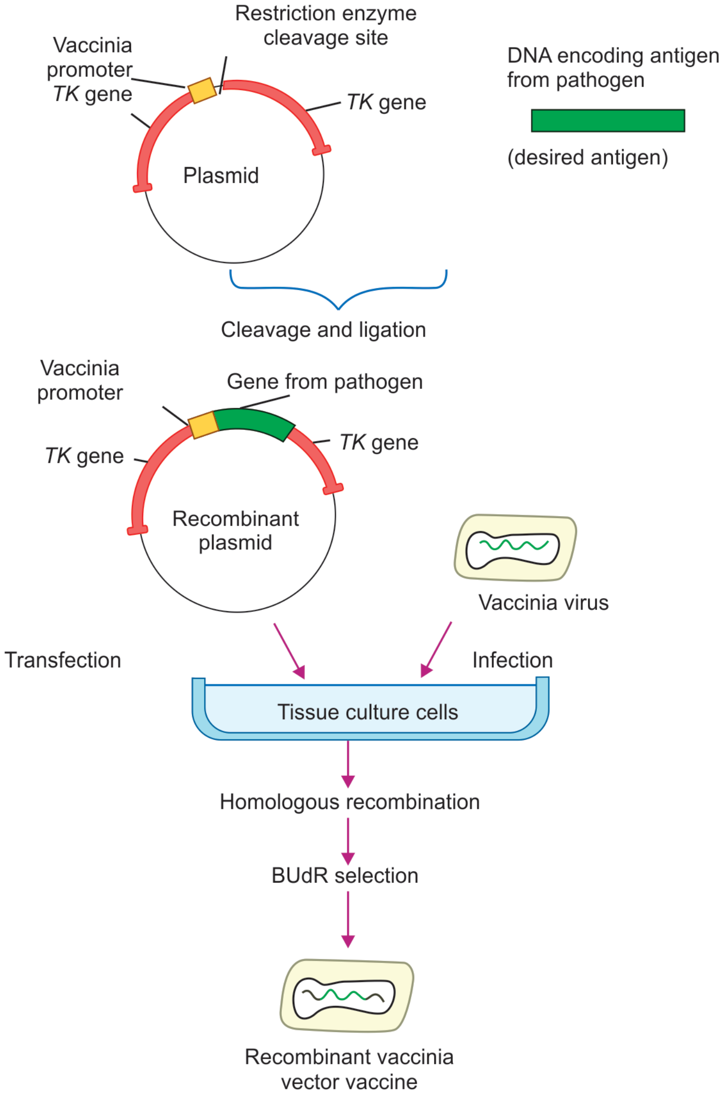
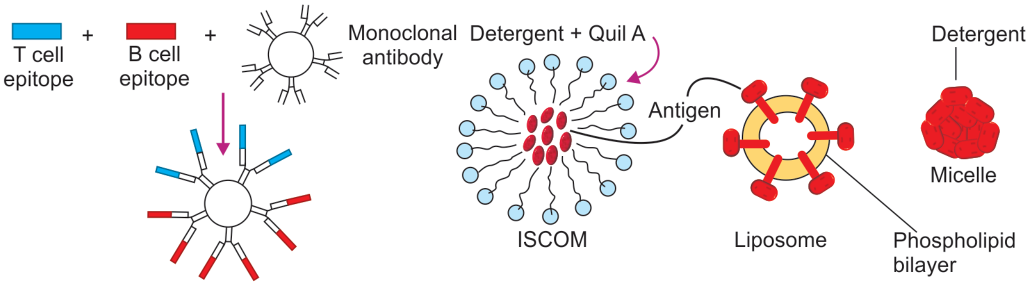

Vaccinia
promoter
TK gene
Restriction enzyme
cleavage site
TK gene
Plasmid
DNA encoding antigen
from pathogen
(desired antigen)
Cleavage and ligation
Vaccinia
promoter
Gene from pathogen
TK gene
TK gene
Recombinant
plasmid
Vaccinia virus
Transfection
Infection
Tissue culture cells
Homologous recombination
BUdR selection
Recombinant vaccinia
vector vaccine

**Fig. 17.1:** Production of vaccinia vector vaccine. The gene that encodes the desired antigen (green) is inserted into a plasmid vector adjacent to a vaccinia promoter (yellow) and flanked on either side by the vaccinia thymidine kinase *(TK)* gene (reddish orange). When tissue culture cells are incubated simultaneously with vaccinia virus and the recombinant plasmid, the antigen gene and promoter by homologous recombination at the site of the non-essential *TK* gene, resulting in a *TK* recombinant vaccinia virus, selected by addition of 5-bromodeoxyuridine (BUdR), which kills TK cells.

T cell epitopes. Secondly, for better cytolytic T lymphocyte (CTL) response, the vaccine must be delivered intracellularly, so that the peptides are processed and presented with class-I major histocompatibility complex (MHC) molecules. A number of new techniques are being applied to develop multivalent vaccines that can present multiple copies of a given peptide or mixture of peptides to the immune system (Fig. 17.2).

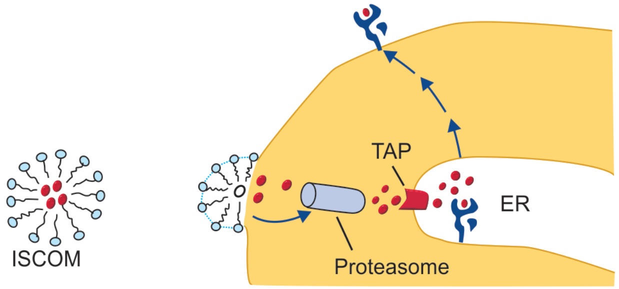
One approach is to prepare solid matrix antigen-antibody (SMAA) complexes by attaching monoclonal antibodies (mAbs) to particulate solid matrices and saturating the antibody with desired antigen. The formed complexes are used as vaccines. By attaching different monoclonal antibodies to the solid matrix, it has become possible to bind mixture of peptides or proteins, composing immunodominant B and T cell epitopes. These multivalent complexes produce marked humoral and CMI. Their particulate nature provides better immunogenicity.

Another approach to produce multivalent vaccines is to use detergent to incorporate protein antigens or synthetic antigenic



A Solid matrix Ab-Ag complex

B Detergent extracted membrane antigen peptides



C ISCOM delivery of antigen into cell

**Figs 17.2A to C**: Multivalent subunit vaccines. **A.** Solid matrix antibody-antigen complexes can be designed to contain synthetic peptides representing both T cell epitopes and B cell epitopes; **B.** Protein micelles, liposomes and immunostimulating complexes (ISCOMs) can all be prepared with extracted antigens or antigenic peptides. In micelles and liposomes, the hydrophilic residues of the antigen molecules are oriented outward. In ISCOMs, the long fatty-acid tails of the external detergent layer are adjacent to the hydrophobic residues of the centrally located antigen molecules; **C.** ISCOMs and liposomes can deliver antigens inside cell, so they mimic endogenous antigens. Subsequent processing by the cytosolic pathway and presentation with class-I MHC molecules induces a cell-mediated response. (TAP, transporter associated with antigen processing; ER, endoplasmic reticulum).

peptides into protein micelles, into lipid vesicles (liposomes) or into immunostimulating complexes (ISCOMs).

Membrane proteins from various pathogens such as influenza, measles and hepatitis B viruses have been incorporated into micelles, liposomes and ISCOM and are currently addressed as potential vaccines.

## STUDY QUESTIONS

### Essay Questions

1. Classify the common vaccines, which are in use for human beings. How do vaccines and toxoids differ?
2. List the major differences among vaccines. What are the major benefits and hazards of active immunization?

### Short Notes

1. Live vaccines.
2. Polysaccharide vaccines.
3. Recombinant antigen vaccines.
4. Subunit vaccines.
5. DNA vaccines.

## SUGGESTED READING

1. Black JG. Microbiology: Principles and applications, 3rd edition. USA: Printice Hall College div; 1996.
2. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
3. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
4. Goldsby RA, Kindt Thomas J, Osborn Barbara A. Kuby Immunology, 6th edition. New York: WH Freeman and Company; 2007.
5. Jawetz, Melnick and Adelberg's Medical Microbiology, 25th edition. Lange Basic Science, USA: McGraw Hill; 2010.
6. Male David, Brostoff Jonathan, Roth David, et al. Immunology, 7th edition. Mosby Elsevier; 2006.
7. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. Ist Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.
8. Tortora Gerard J, Funke Berdel R, Case christine L. Microbiology: an introduction, 10th edition. USA; 1998.

# Immunity in Parasitic, Viral, Bacterial and Fungal Infections

# 18

## IMMUNITY IN PARASITIC INFECTIONS

### General Features

Parasites infect, very large number of people and present major medical problems, especially in tropical countries. The diseases caused are diverse and the immune responses, which are effective against the different parasites vary considerably. Parasitic infections do, however, share a number of common features.

Protozoan parasites and the worms are considerably larger than the bacteria and viruses, not only more in quantity, but also in variety. The parasites, unlike bacteria and viruses, undergo a life cycle in the host and exhibit different antigenicity at different stages of life, besides some species also change their surface antigens, a process known as antigenic variation *(Trichinella spiralis)*. Protozoa evolve different mechanisms to enter inside the cell to have their intracellular existence. The invasive merozoite attached itself to the receptor on erythrocytes and uses the cell. On the other hand, *Leishmania* species use the complement receptor on macrophage to be engulfed by macrophage, where ultimately they reside and multiply.

The host resistance to parasite infection may be genetic and controlled by immune response genes situated in the major histocompatibility complex class II (MHC II) area. Certain individuals are genetically less susceptible to certain parasites. People, with sickle-cell trait, are resistant to malaria. For example, merozoites of the malaria parasite *(Plasmodium vivax)* use a particular blood group substance on to erythrocyte surface, the Duffy antigen, as a receptor to their entry into the cell. Certain African populations lack this antigen and are totally resistant to infection by parasite.

Parasitic infections are generally chronic and show marked host specificity. For example, the malarial parasites of birds' rodents and man can multiply only in its own particular kind of host. Some exceptions are there. The tapeworm of pig can also infect humans.

### Effector Mechanisms

After the entry of the parasite into the host, before it faces the specific immune response, it has to overcome the host's pre-existing defense mechanisms. Complement plays role in eliminating or causing lysis of many parasites, including certain adult worms and infective larva of *T. spiralis,* schistosomules of *Schistosoma mansoni*. Natural killer (NK) cell also may be active in imparting innate immunity against parasitic infection initially.

Various kinds of effector cells such as macrophages, neutrophils, eosinophils and even platelets helps to defend the host against the invasion of the parasites and act to control the multiplication and spread of the parasites, already in the residence. They act as the cells of the first line of defense.

### *Macrophage*

Apart from acting as antigen processing and presenting cells in initiating immune response, macrophage affects the course of parasitic infection in two ways:

1. They secrete molecules, which act to regulate the inflammatory response. Interleukin-1 (IL-1), tumor necrosis factor (TNF) and colony-stimulating factor (CSF) may enhance immunity by activating other cells or stimulating their proliferation. Macrophage releases pro-staglandins, which may be immunosuppressive.
2. They act as effector cells, which inhibit the multiplication of the parasite or they may destroy them.

Activation of macrophages is a general feature of the early stages of infection. Activation of macrophages is brought about by the cytokines produced by T helper ($T_h1$) cell [interferon-γ (IFN-γ), granulocyte-macrophage (GM)-CSF, IL-3, etc.]. Some products of the parasite such as *Trypanosoma brucei* and malarial parasites can cause activation of macrophage, independent of T cells, perhaps directly or by inducing macrophages to secrete TNF-α, which then activate other macrophages.

*Granuloma formation in liver and fibrous encapsulation:* In some parasitic infections in which the immune system cannot completely eliminate the parasite, the body reduces damage by walling of the parasite behind a capsule of inflammatory cells. This reaction is T cell dependent, is a chronic cell-mediated immune response to released antigen. Macrophages accumulate, release fibrogenic factor of granuloma tissue. This sort of granuloma formation occurs in schistosomal infection in which the eggs are trapped in liver.

*Phagocytosis:* It is one of the primary functions of macrophage and is important in the defense against smaller parasite invaders. The effectiveness is markedly enhanced by opsonization of the organisms to be ingested. In addition, activated macrophages may express more Fc and C3 receptors, which also tend to enhance their phagocytic function. African trypanosomes are quickly taken up by phagocytic cells in the liver.

*Parasite killing properties:* Macrophages secrete scores of soluble factors, many of which can be cytotoxic and consequently they can also kill parasites by process that do not depend upon ingestion.

Activated macrophages are important in the control of infections caused by, for example, *Trypanosoma cruzi, Leishmania and Plasmodium* species. Macrophages are capable of killing not only, relatively small intracellular parasite such as erythrocytic stage of malarial parasite, but also, when activated, larger parasite such as the larval stage of schistosomes. They can also act as the killer cells by antibody-dependent cell-mediated cytotoxicity (ADCC). Specific immunoglobulin G (IgG) and IgE for instance can enhance their ability to kill schistosomules.

### *Granulocytes*

*Phagocytic properties:* They can kill by both oxygen ($O_2$)-dependent and $O_2$-independent mechanisms. They produce more intense oxidative bursts than macrophages and their secretory granules contain highly cytotoxic protein.

*Extracellular destruction:* The neutrophils also can be activated by cytokines (IFN-γ, GM-CSF, TNF-α). Extracellular destruction of *T. cruzi* is mediated by superoxide and hydrogen peroxide.

*Neutrophils:* They also bear Fc receptor and receptor for complement that renders them (*S. mansoni* and *T. spiralis*) effective participants in ADCC.

### *Eosinophils*

Infiltration of eosinophils and the production of high level of IgE are the common consequences of infection by parasitic worms and the eosinophils appear as major effector cell against helminths. It has been argued that the eosinophil has evolved, especially, as a defense against the tissue stage of the parasites that are too large to be phagocytosed (e.g. helminths). The IgE-dependent mast cell reaction has evoked, primarily, to localize eosinophils near the parasite and then enhances the antiparasitic function through eosinophilic chemotactic factor (ECF). The increase in the number of eosinophils in schistosomiasis and ascariasis is also T cell dependent. The recruitment of eosinophils is mediated by a specific factor, eosinophilic stimulation promoter, which is a product of T cell. The eosinophils function in a various ways:

1. In association with specific antibody, they kill the worm by ADCC. Damage to helminths (schistosomulae) can be caused by the major basic protein liberated after degranulation.
2. Eosinophils are less phagocytic than neutrophils, but like neutrophils they can kill parasites by both $O_2$-dependent and $O_2$-independent mechanisms.
3. Enzymes released from the granules exert a controlling effect on the substances released from the mast cells. The mast cell-derived factors are important in controlling the permeability of the local blood vessels and inflammation at the site of infection.

### *Mast Cells*

Mast cell mediators enhance the activity of other effector processes in worm infection:

1. They interact with eosinophils as described.
2. They play an important role in accelerating the expulsion of worms.

In response to local release of antigen, T cells produce cytokines, which induce a vast increase in the number of inflammatory cells including monocytes, mucosal mast cells and goblet cells.

Mast cells also promote enhanced secretion of mediators and of mucus and provide help for the accumulation of IgG, IgA and IgE. Mast cell products including proteinase, cause change in permeability of the gut and shedding of the epithelium, which may help ejecting certain protozoan parasites.

### *Platelets*

Platelets are capable of killing various types of parasites, including *Schistosoma, Toxoplasma gondii and T. cruzi*. Further, they also bear Fc receptors for IgE on their surface membrane by which they mediate ADCC associated with IgE.

### *T Cells*

The T cells play important role in counteracting protozoan infections. It has been shown that nude mice, depleted of Tcells, have reduced capacity to control trypanosomal and malarial infection. The transfer of splenic cells, especially T cells from immune animals, gives protection against most parasitic infection.

Both $CD4^+$ and $CD8^+$ cells are needed for protection against some parasites. The type of T cells responsible for controlling infection varies depending on the type of parasite, the stages of infection and the cytokines they produce. The $CD4^+$ and $CD8^+$ T cells protect against the different phases of *Plasmodium* infection. The $CD4^+$ cells mediate immunity against blood stage. The $CD8^+$ cells protect against the liver stage. The $CD8^+$ cells act in two ways. They secrete IFN-$\gamma$, which inhibits the multiplication of the parasite in the liver cells and also they destroy hepatocytes by cytotoxic action, because of the presence of

MHC I in hepatocytes. The CD8$^+$ cells cannot affect blood stage parasite as the erythrocytes do not possess MHC I molecule.

The immune response against *T. cruzi* and *T. gondii* depend not only upon CD4$^+$ and CD8$^+$ cells, but also NK cells and antibody production.

The CD4$^+$ cells act in different ways in different infections. $T_h$ cells are of two types $T_h1$ and $T_h2$. The $T_h1$ and $T_h2$ cytokines are mutually antagonists. $T_h1$ and $T_h2$ cells both counter malarial infections. $T_h1$ cells act against the liver stage of malaria. Administration of IFN-γ ($T_h1$ cytokines) directly to chimpanzee, infected with sporozoites of vivax diminishes parasitemia. $T_h2$ cells through IL-4 activate B cells to produce specific antibody. Elimination of blood stage of malaria occurs in the spleen via the activated effector cells and by antibody-dependent cytotoxicity.

T helper type 1 subset enhances protective response against intracellular protozoa. $T_h1$ subsets produce IFN-γ, which activate macrophages to kill protozoa that live within them *(L. donovani, T. gondii)*. It also enhances the effector responses against other parasites.

Both $T_h1$ and $T_h2$ responses are important in helminthic infection. IgE and eosinophils play vital role in the immunity against most of the helminthic infections and depend on the cytokines production by $T_h2$ cells. The role of $T_h1$ cells also cannot be ignored. In schistosomiasis, the immune responses differ in mice, rats and in human beings. $T_h2$ response is more important as resistance after drug treatment is correlated with the production of IgE. In the mouse, $T_h1$ cells and IFN-γ are needed for vaccine induced protection and $T_h2$ cells are associated with egg-related immunopathology. The switch to $T_h2$ is triggered by egg-related antigens.

The secretion of IFN-γ by $T_h1$ cells activates effector cells that destroy lung-stage larvae, via the production of nitric oxide, but when the adult schistosomes *(S. mansoni)* lay eggs, a soluble antigen is released. The antigen reduces $T_h1$ cells function, as well as the level of IFN-γ and increases $T_h2$ cell production and IL-5.

In some parasites, when the immune system will fail to completely eliminate the organism, the damage to body is reduced by granuloma formation by inflammatory cells and fibroblast surrounding the remnant of parasite. This $T_h1$-mediated reaction is a chronic cell-mediated response to locally released antigen and is mediated by cytokines, especially TNF and IFN-γ.

The immune response induced against various worms depend upon the anatomical sites (gut, tissue), where they are present and also the antigens they gain, while pass through their life cycles.

### *Antibodies*

In addition to the rise in specific antibodies, many parasitic infections provoke non-specific hypergammaglobulinemia. While specific responses are mostly T cell dependent, much of the non-specific antibody production is probably due to antigens released from the parasites acting as B cell mitogens.

Specific antibody is particularly important in the control of extracellular parasites. The antibody is effective in preventing the reinvasion of the cells by blood-borne parasites, but ineffective once the parasite enters the host cells. The mechanism by which specific antibody controls the parasitic infections are summarized (Fig. 18.1).

Antibody is also involved in ADCC. Cytotoxic cells such as macrophages, neutrophils and eosinophils adhere to worms coated with antibody by means of Fc and C3 receptors. Damage to schistosomes caused by the major basic protein (MBP) of the eosinophils crystalloid core. The release of MBP into a small space between the

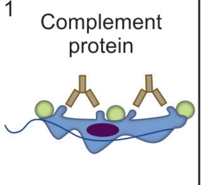
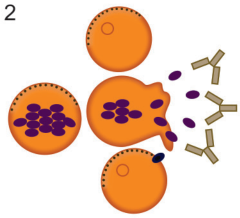
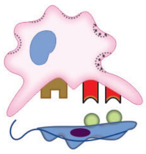
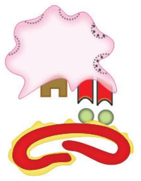
| Parasite | *Plasmodium* sporozoite, intestinal worms, trypanosome | *Plasmodium* sporozoite and merozoite, *Trypanosoma cruzi*, *Toxoplasma gondii* | *Plasmodium*, *Trypanosoma* | Schistosomes, *Trichinella spiralis*, Filarial worm larvae |
|---|---|---|---|---|
| Mechanism | 1  | 2  | 3  | 4  |
| Effect | Direct damage or complement-mediated lysis | Prevents spread by neutralizing attachment site, prevents escape from lysosomal vacuole, prevents inhibition of lysosomal fusion | Enhancement of phagocytosis | Antibody-dependent cell-mediated cytotoxicity (ADCC) |

**Fig. 18.1: 1.** Antibody-mediated defense of parasitic infections, direct damage: Antibody activates the classical complement pathway, causing damage to the parasite membrane and increasing susceptibility to other mediators; **2.** Neutralization: Parasites such as *Plasmodium* species spread to new cells by specific receptor attachment; blocking the merozoite binding site with antibody prevents attachment to the receptors on the erythrocyte surface and hence prevents further multiplication; **3.** Enhancement of phagocytosis: Complement C3b deposited on parasite membrane opsonizes it for phagocytosis by cells with C3b receptors (for example, macrophages). Macrophages also have Fc receptors; **4.** Eosinophils, neutrophils, platelets and macrophages may be cytotoxic for some parasites when they recognize the parasite via specific antibody (ADCC). The reaction is enhanced by complement.

eosinophils and the schistosomes surface localizes its action on the teguments of the worm and kill them.

## Escape Mechanisms

There are various mechanisms, how the parasites evade the host immune system and establish infections.

### Intrinsic Resistance

*Anatomical inaccessibility (seclusion):* Many parasites are protected from the host defense by their anatomical inaccessibility. For example, intracellular parasites avoid the effect of antibody being in accessible (*T. cruzi, Plasmodium* species and leishmanial species). Intracellular parasites residing inside the macrophages also evoke different strategies of avoiding being killed by $O_2$ metabolites and lysosomal enzymes. Some parasites *(Entamoeba histolytica)* are protected by changing to cystic form.

*Ability to resist destruction by complement: L. donovani* offers more resistance to complement-mediated lysis than *L. tropica.*

### Avoidance of Recognition

Certain parasites undergo antigenic variation (African trypanosomes) and change the antigens of their surface coat. Each variant possess antigenically distinct glycoproteins, which form its surface coat. The surface coat, presumably, protects the underlying surface membrane from the host's defense mechanisms. Malaria parasites also show antigenic variation.

Other parasites, for example, *Schistosoma* species acquire a surface layer of host's antigens, so that the host defense does not distinguish them from the self.

### *Suppression of the Host's Immune Response*

1. Parasites can cause disruption of lymphoid cells or tissue directly (the soluble lymphotoxic factor of *T. spiralis*). *Schistosoma* can cleave a peptide from IgG.
2. Soluble parasite antigens, which can occur in enormous quantities, may reduce the effectiveness of host's response by a process known as immune distraction.
3. Non-specific immunosuppression is a universal phenomenon of parasitic infection as a whole.
4. Macrophage dysfunction may occur due to antigenic load. Antigen processing capacity is reduced. There will be decreased production of IL-1. Macrophage also releases prostaglandins, which suppress the immune reaction.
5. Specific immunosuppression may occur as that happens in leishmaniasis. In this case, there is immunosuppression of T cell reactivity, which is harmful for the host because protection depends on cell-mediated immunity (CMI) (Fig. 18.2).

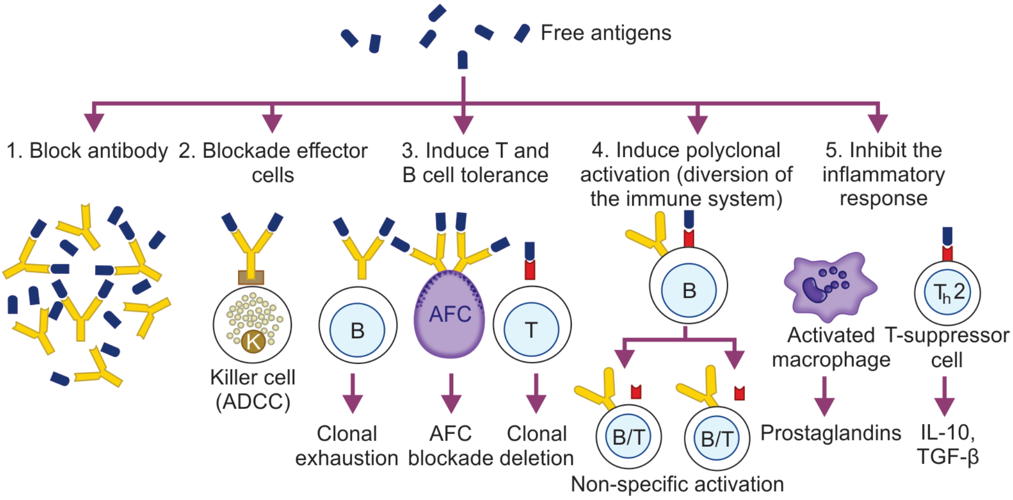

Free antigens
1. Block antibody
2. Blockade effector cells
3. Induce T and B cell tolerance
4. Induce polyclonal activation (diversion of the immune system)
5. Inhibit the inflammatory response
K
Killer cell (ADCC)
B
AFC
T
Clonal exhaustion
AFC blockade
Clonal deletion
B
B/T
B/T
Non-specific activation
Activated macrophage
Prostaglandins
$T_h2$
T-suppressor cell
IL-10, TGF-β

**Fig. 18.2:** Free antigens can: **1.** Combine with antibody and divert it from the parasite. The variant surface glycoprotein of *Trypanosoma brucei* and the soluble antigens of *Plasmodium falciparum,* which are also polymorphic and contain repetitive sequences of amino acids, are thought to act in this way as a smoke-screen or decoy; **2.** Blockade effector cells, either directly or as immune complexes. Circulating complexes, for example, are able to inhibit the action of cytotoxic cells active against *Schistosoma mansoni*; **3.** Induce T or B cell tolerance, presumably by blockage of antibody-forming cells (AFC) or by depletion of the mature antigen specific lymphocytes through clonal exhaustion; **4.** Cause polyclonal activation. Many parasite products are mitogenic to B or T cell and the high serum concentrations of non-specific IgM (and IgG) commonly found in parasitic infections probably result from this polyclonal stimulation. Its continuation is believed to lead to impairment of B cell function, the progressive depletion of antigen-reactive B lymphocytes and thus immunosuppression; **5.** Activate T cells, especially $T_h2$ cells or macrophages or both, to release immunosuppressive molecules.

### Immunopathological Consequences of Parasitic Infections

Apart from the directly destructive effects of some parasites and their products, many immune responses, themselves, have some pathological effects.

1. The IgE liberated in response to worm infections have serious effects on the hosts through mast cell mediators.

   *Examples:*

   a. Anaphylactic shock may occur when the hydatid cyst ruptures.
   b. Asthma like reaction occurs in *Toxocara canis* infection.
   c. Tropical eosinophilia occurs when filarial worms migrate through the lungs.

2. The formation of immune complex is common in parasitic infection.

   *Examples:*

   a. Immune complex may be deposited in the kidney, as in the nephrotic syndrome of quartan malaria.
   b. Immune complex containing parasitic antigen may bind to unparasitized cells and accelerate their phagocytosis by the phagocytic cell of the spleen and liver.

3. Autoantibodies have been detected against red cells, lymphocytes and deoxyribonucleic acid (DNA) (e.g. in trypanosomiasis and malaria).
4. Cross-reacting antigens: Antibodies against parasite may cross-react with host tissues.

   *Example:*

   a. Cardiomyopathy, enlarged esophagus and megacolon that occurs in the Chagas' disease are thought to result from the autoimmune effects of antibody or cytotoxic T cells on nerve ganglia that cross-reacts with *T. cruzi*.

5. There will be increase in the numbers and activity of macrophages and lymphocytes leading to splenomegaly and hepatomegaly in malaria, sleeping sickness, visceral leishmaniasis.
6. Granuloma formation around the worm eggs leads to enlarged liver and fibrosis.
7. Excess production of cytokines may contribute to the sum of the manifestations of the disease. Thus the fever, anemia, diarrhea and pulmonary changes of acute malaria closely resemble the symptoms of endotoxemia and probably caused by TNF-α.
8. Many immunological mechanisms may be combined in their pathological effects to cause anemia in malaria.
9. Non-specific immunosuppression explains, why people with parasitic infections are, especially, perceptible to bacterial and viral infection (measles).

## IMMUNITY IN VIRAL INFECTION

The host response to invading virus depends upon the infectious agents and where it is encountered. In response to virus entry there may not be, always, an overt reaction leading to clinical manifestation. There may be simply, subclinical infection, which would protect the individual from later exposure. A number of specific immune effector mechanisms together with non-specific defense mechanism play role in eliminating an infective virus (Table 18.1). At the same time the virus also finds out ways to subvert the defense mechanism and establish the infection.

### Innate Immune Response

The interferon-alpha and beta (IFN-α and -β) and NK cells play vital role in imparting innate immune response to viral infection. IFN-α and IFN-β are produced in response to the presence of viruses and certain intracellular bacteria. Double-stranded ribonucleic

**Table 18.1** Mechanisms of humoral and cell-mediated immune responses to viruses

| Response type | Effector molecule or cell | Activity |
|---|---|---|
| Humoral | Antibody (especially, secretory IgA) | Blocks binding of virus to host cells, thus preventing infection or reinfection |
| | IgG, IgM and IgA antibody | Blocks fusion of viral envelope with host-cell plasma membrane |
| | IgG and IgM antibody | Enhances phagocytosis of viral particles (opsonization) |
| | IgM antibody | Agglutinates viral particles |
| | Complement activated by IgG or IgM antibody | Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex and direct antiviral activity |
| Cell mediated | IFN secreted by $T_h$ or $T_c$ cells | Kill virus-infected self-cells |
| | Cytotoxic T lymphocytes (CTLs), NK cells and macrophages | Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC) |

acid (dsRNA) may be the important inducer. Macrophages, monocytes and fibroblasts are also capable of synthesizing these cytokines. IFN-α and IFN-β can induce an antiviral response or resistance to viral replication by binding to IFN receptors. Following binding of these IFNs with IFN-receptor, there is induction of the synthesis of both 2-5 oligoadenylate synthetase, (2-5A synthetase) and protein kinase-RNA (PKR). The action of 2-5A synthetase results in the activation of ribonucleaseL (RNaseL), which can degrade messenger-RNA (mRNA) protein kinase, inactivates the translation initiation factor by phosphorylating it. Both pathways thus, result in the inhibition of protein synthesis and there by, effectively block viral replication (Fig. 18.3).

Certain cell-mediated reactions are also the part of the innate defenses against viral infections. NK cells play important role in this aspect. The formation of a close conjugate between the NK cell and the target, induces the effector cell to produce toxic molecules, which bring about the destruction of target cell. Natural killing is increased by IFNs, both the number of effector cells and their killing potential. Therefore, these two innate defense mechanisms appear to work together to protect the host from viral infection.

## Humoral Immunity

Humoral immunity are various ways, how the antibodies against the viral components protect the host. Antibodies have no action against the latent viruses, as well as the viruses those spread from cell to cell. Small amount of antibody in the blood, can neutralize the virus before it reaches its target cells in the nervous system. Most viruses express surface receptor molecules that enable them to initiate infection by binding to these molecules on the complementary part on the tissues [sialic acid residues in cell membrane glycoprotein and glycolipid for influenza virus, intercellular adhesion molecules (ICAMs) for rhinovirus, type 2 complement receptors on B cell for Epstein-Barr (EB) virus]. The antibodies block the receptor molecules on the viruses, thus, preventing their attachment to the complementary tissues. Secretory IgA can neutralize viruses by similar mechanism on mucosal surfaces.

In some cases, antibodies may block viral penetration by binding to epitope that are necessary, to mediate fusion of the viral envelope with the plasma membrane. Antibodies also can work at stages after penetration. Uncoating with its release of viral nucleic acid, into the cytoplasm, can be inhibited if the virion is covered by antibody. If the induced antibody is of complement-activating isotypes, lysis of enveloped virions can ensue. Antibody can also cause aggregation of virus particles, thus, limiting the spread of the infectious particles and forming a complex that is readily phagocytosed. Antibody and complement can also function as an opsonizing agent to facilitate Fc or C3b receptor-mediated phagocytosis of the viral particles.

Humoral immunity does play a major protective role in polio and in number of other viral infections. Passively administered antibody can protect humans against several infections including measles, hepatitis A and B

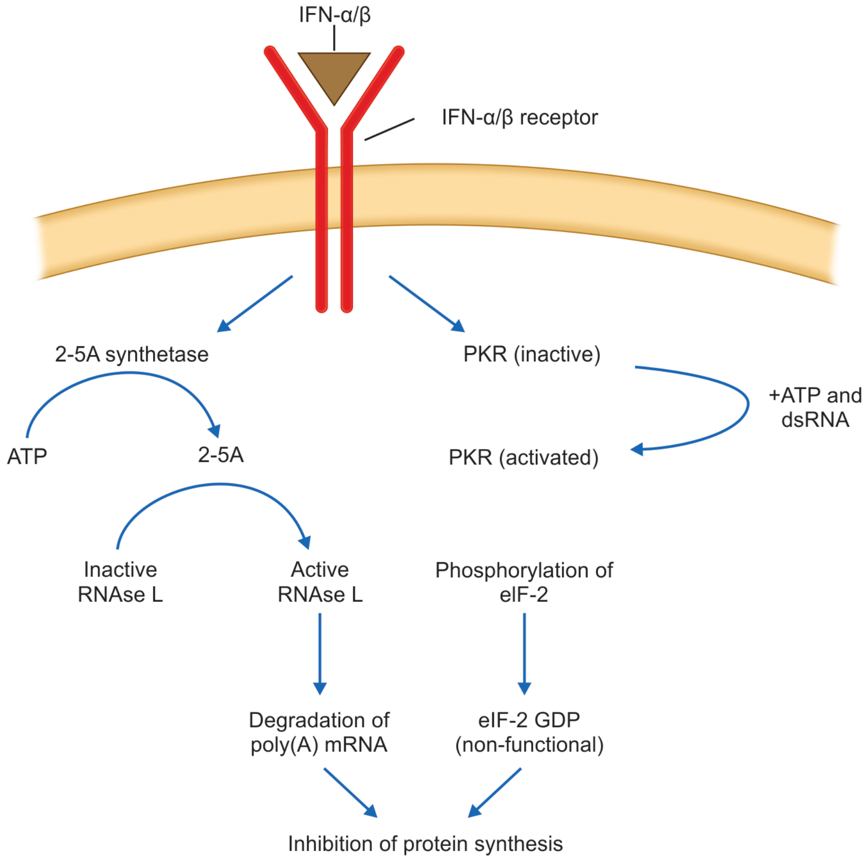

IFN-α/β
IFN-α/β receptor
2-5A synthetase
PKR (inactive)
+ATP and dsRNA
ATP
2-5A
PKR (activated)
Inactive RNAse L
Active RNAse L
Phosphorylation of eIF-2
Degradation of poly(A) mRNA
eIF-2 GDP (non-functional)
Inhibition of protein synthesis

**Fig. 18.3:** Induction of antiviral activity by IFN-α and -β. These IFNs bind to the IFN receptor, which in turn induces the synthesis of both 2-5A synthetase and protein kinase RNA (PKR). The action of 2-5A synthetase results in the activation of RNAse L, which can degrade mRNA. PKR inactivates the translation initiation factor eIF-2 by phosphorylating it. Both pathways thus result in the inhibition of protein synthesis and thereby effectively block viral replication.

and chickenpox, if given before or very soon after exposure. The immunity to many viral infections is lifelong.

## Cell-mediated Immunity

As long as the virus is extracellular and the infection is not established, the antibody plays major role either eliminating the virus by different mechanisms or preventing the entry by blocking the receptor site. Antibody plays no role, once the virus is intracellular and the DNA of virus is integrated into the host DNA.

Once the infection is established CMI is imperative to deal with the virus. In general, $CD8^+$ [cytotoxic T lymphocytes (CTL) cells] and $CD4^+$ ($T_h1$) cells are the main cell types, which take part in the defense mechanisms. Activated $CD4^+$ ($T_h1$) cells produce a number of cytokines such as IFN-α, IL-2 and TNF-β, which defend against virus infection directly or indirectly. IFN-α acts directly on the virus infected cell and produce antiviral state. IL-2 activates CTLs and potentiates the lytic action on viral infected cells. Both IFN-γ and IL-2 activate NK cells, which play important role in causing lysis of the viral infected cells by ADCC mechanism in the beginning of the infection, when specific CTLs have not developed. The role of CTLs in defense against viruses is demonstrated by the ability of virus specific CTLs to confer protection for the specific virus on non-immune recipient by adoptive transfer.

## Viral Evasion of Host Defense Mechanism

Despite adequate immune response produced against the viral components, virus evades the defense mechanism of the host and establish infection (Fig. 18.4).

In many viruses, additional proteins are produced that interfere at various levels with specific and non-specific defenses. The advantage of such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses (Fig. 18.4). There are certain viruses, which evolve the strategies to evade the action of IFN-α and IFN-β. These include hepatitis 'C' virus, which has been shown to overcome the antiviral effect of IFNs by blocking the action of PKR. Herpes simplex virus (HSV) both I and II, evade host defense mechanism by inhibition of antigen presentation by infected host cells. Both HSV-I and HSV-II express an immediate early protein called infected cell protein (ICP) 47, which very effectively inhibits the human transporter molecule (TAP) needed for antigen processing. The targeting on MHC molecule is not unique to HSV. In adenovirus and cytomegalovirus (CMV) also there is down regulation of MHC I expression inhibiting antigen presentation to $CD8^+$ T lymphocytes. In some viral infections such as CMV, measles and human immunodeficiency virus (HIV) infection, there is also reduction of MHC II molecule expression on the surface of antigen-presenting cell (APC), thus blocking the function of antigen-specific antiviral $T_h$ cells.

Antibody-mediated destruction of the virus depends on the activation of the complement leading to lysis or phagocytosis following opsonization, but certain viruses develop strategies, to overcome complement-mediated destruction. For example, vaccinia virus secretes protein that binds to C4b component of the complement and inhibit the classical complement pathway. Similarly, the glycoprotein component of HSV virus binds to C3b component inhibiting both classical and alternative pathway.

Many other viruses evolve the host's defense mechanism by adopting, antigenic variation. The best example is influenza virus, which undergoes antigenic variation, producing a different strain. The antibody produced against earlier strain becomes ineffective. An-

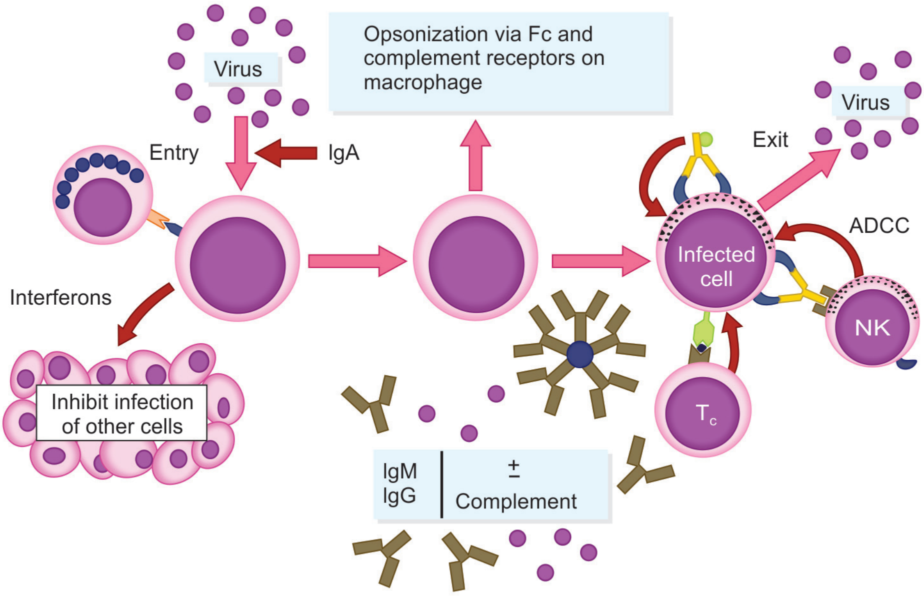

Virus
Opsonization via Fc and complement receptors on macrophage
Virus
Entry
IgA
Exit
ADCC
Infected cell
NK
Interferons
$T_c$
Inhibit infection of other cells
IgM
IgG
±
Complement

Fig. 18.4: Entry of virus at mucosal surfaces inhibited by IgA. Following the initial infection, the virus may spread to other tissues via bloodstream, IFNs produced by the innate (IFN-α and IFN-β) and adaptive (IFN-γ) immune responses make neighboring cells resistant to infection by spreading virus. Antibodies are important in controlling free virus. Whereas T cells and NK cells are effective at killing infected cells (ADCC, antibody-dependent cellular cytotoxicity).

tigenic variation in rhinoviruses (the causative agent of common cold) is responsible for inability in producing vaccine against common cold. Antigenic variation also, is very common in HIV due to regular mutations, is the greatest impediment in preparing vaccine.

A large number of viruses evade host's immune mechanism by causing generalized immunosuppression. Among them are mumps, measles, EB virus and HIV. Some viruses, directly destroy the lymphocytes and macrophages (HIV causing lysis of $CD4^+$ cells). In other cases, the immunosuppression may be due to cytokine imbalance. EB virus produces a protein (BCRF1), which has got similar action as IL-10, which suppresses $T_h1$ action leading to decreased level of IFN-γ and IL-2.

## IMMUNITY IN BACTERIAL INFECTIONS

Bacteria enter the body either through a number of natural routes (e.g. the respiratory tract, the gastrointestinal tract and the genitourinary tract) or through broken skin and mucous membrane. Different levels of host defense are enlisted depending on the number of organisms and their virulence. If the inoculum size is small and the virulence of the organisms are low, they can be eliminated by phagocytic cells of the innate defense system. On the other hand, if the size of the inoculums is larger and the organisms are more virulent, then the role of specific immune response comes. Usually, when the bacteria are extracellular, they are eliminated by antibody-mediated defense. For intracellular bacteria, CMI plays important role.

## Host Defenses

Very few organisms can penetrate the intact skin, because of various innate defense mechanisms. Whenever, the bacteria gain access to the tissues, their ability to fight the organisms and to eliminate depends upon the immune response generated against the microbial antigens. In most cases, the immune reponse is generated against the components of the bacteria and the molecules secreted by them. Immune response is also generated against the organ of motility (flagella), the organ of adhesion (fimbriae) and also the capsules. Specific antibodies to flagella and fimbriae also affect their ability to function properly. Antibodies also can inactivate various bacterial enzymes and toxins.

The bacteria after successfully evading the innate defense mechanisms (mechanical barrier, antibacterial substances, phagocytosis, etc.), proliferate in the tissue liberating the toxic products, which trigger inflammation. The resulting increased vascular permeability leads to the exudation of serum, which contains complement components, antibodies, clotting factors, as well as phagocytic cells. Chemotactic factors attract the phagocytic cells to the site of inflammation. Anaphylatoxins (C3a, C5a) generated by complement activations, further increase the vascular permeability increasing blood flow to the area.

Two types of adaptive immunity play roles against bacterial infection. They are humoral immunity and CMI.

## Humoral Immunity

Attachment and invasion are important processes, which pathogenic bacteria adopt to establish the infection. Certain antibodies such as secretory IgA, interfere with the attachment molecule (agresin) and prevent colonization of pathogenic bacteria. Many organisms produce disease through their exotoxins (diphtheria, tetanus, botulism, etc).

Antibodies acquired by either immunization or previous infection or given passively, neutralize the bacterial exotoxins. Subsequently the toxin-antitoxin complexes are phagocytosed. Many bacterial exotoxins are enzymes. The antibody against enzymes interferes with the ability of the enzyme to interact with substrates.

Antibody can interfere the normal functioning of bacteria, if in various ways when it binds directly to bacteria. Direct binding affects the activity of specific transport systems, there by depriving the bacteria of its energy supply and other essential chemicals. Invasion of the bacteria can also be inhibited by restricting the motility, when antiflagellar antibodies are produced. Specific antibody can agglutinate the bacteria, thus restricting the dissemination of the organism.

Antibody that binds to accessible antigens on the surface of a bacterium together with C3b component of complement, act as an opsonin and increases the phagocytosis and clearance of the bacterium (Fig. 18.5).

In some bacteria, notably gram-negative bacteria, complement activations can lead directly to the lysis of the organism. Antibody-mediated complement activation also produce certain localized effector molecules, which help to amplify the inflammatory response. For example, the complement split products (C3a, C4a and C5a) act as anaphylatoxin to induce mast cell degranulation and thus, vasodilation and extravasation of neutrophils and lymphocytes from blood to tissues.

## Cell-mediated Immunity

Ultimately, all bacteria will be engulfed by macrophages either to kill the bacteria or to remove after extracellular killing. The microbial products (muramyl dipeptide and trehalose dimycolate) and chemotactic factors (formyl methionyl peptide) are the

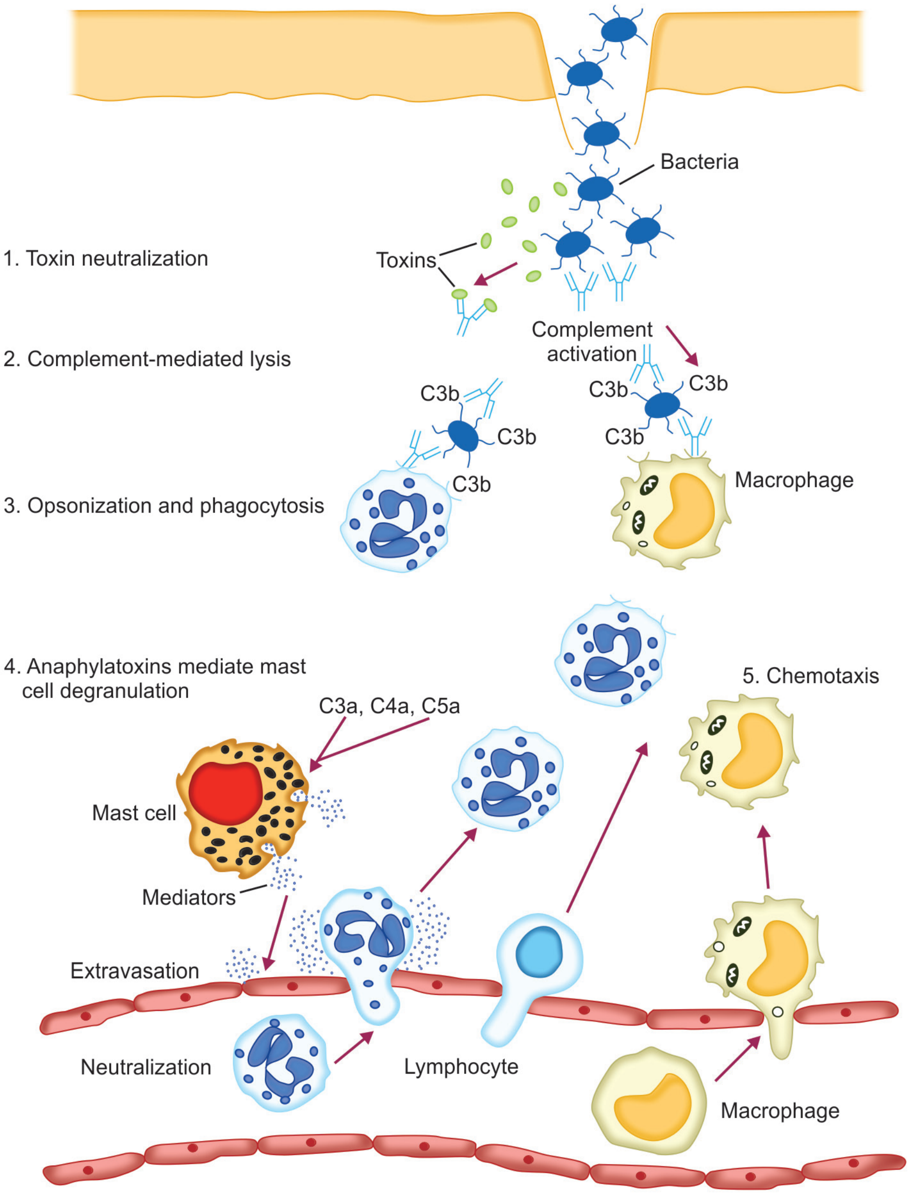

Bacteria
1. Toxin neutralization
Toxins
2. Complement-mediated lysis
Complement activation
C3b
C3b
C3b
C3b
C3b
C3b
3. Opsonization and phagocytosis
Macrophage
4. Anaphylatoxins mediate mast cell degranulation
C3a, C4a, C5a
5. Chemotaxis
Mast cell
Mediators
Extravasation
Neutralization
Lymphocyte
Macrophage

**Fig. 18.5:** Antibody-mediated mechanisms for combating infection by extracellular bacteria. **1.** Antibody neutralizes bacterial toxins; **2.** Complement activation on bacterial surface leads to complement-mediated lysis of bacteria; **3.** Antibody and the complement split product C3b bind to bacteria, serving as opsonins to increase phagocytosis; **4.** C3a and C5a, generated by antibody-initiated complement activation, induce local mast cell degranulation, releasing substances that mediate vasodilation and extravasation of lymphocytes and neutrophils; **5.** Other complement split products are chemotactic for neutrophils and macrophages.

stimuli to activate macrophages and monocytes. The endotoxin present in the cell wall of gram-negative bacteria and various carbohydrate polymers, such as β-glucans, are also potent macrophage activators.

While innate immunity as well as humoral immunity are not very effective against intracellular bacterial pathogens, intracellular bacteria can activate NK cells, which inturn provide early defense against intracellular bacteria. Intracellular bacteria induce a cell-mediated immune response, specifically delayed type of hypersensitivity. The cytokines secreted by CD4$^+$ (T$_h$) cells are important, notably IFN-γ though TNF-β and CSF activate macrophages to kill ingested pathogens more effectively.

### Evasion of Host Defense Mechanisms

Establishment of bacterial infection involves four primary steps. They are:

1. Attachment to host cells.
2. Proliferation.
3. Invasion of host cells.
4. Toxin-induced damage to host cells.

Host defense mechanisms act at each of these steps and many bacteria have evolved ways to circumvent some of these host defenses (Table 18.2).

### Intracellular Bacteria

The bacteria, which can survive and replicate inside the cell are in an advantageous condition, because the antibodies have no access on them. *M. leprae* adopts an intracellular environment, so that they can no longer live extracellularly. *Listeria monocytogenes,* the causative organism of listeriosis, multiply in normal macrophages, but fail to survive in activated macrophages. Listeriosis occurs mostly in immunocompromised subjects, pregnant women and neonate in whom probably the T cell-dependent macrophage activating factors (IFN-γ, TNF-β, etc) are deficient. *Salmonella* species and *Brucella* species can also survive intracellularly. They owe their resistance to glycolipid that is resistant to destruction.

In case of mycobacterial species, there is a waxy cell wall, which is resistant to lysosomal enzymes. *M. tuberculosis* secretes certain molecules, which prevents fusion of lysosome with phagosome, but *M. leprae* escapes phagosome and grow in the cytoplasm. Besides the cell wall of both the mycobacterial species contain lipoarabinomannan, which blocks the action of IFN-γ on macrophages. T$_h$1 represents the major host defense against intracellular bacteria.

In many circumstances, the immune response brings about the destruction of the host tissue. The accumulation of macrophages, in response to the lymphokines (IFN-γ, TNF-β, GM-CSF) secreted by CD4$^+$ cells, will cause formation of granuloma, which will prevent dissemination of the bacteria to other sites of the body.

## IMMUNITY IN FUNGAL INFECTIONS

Fungi are eukaryotes with a rigid cell wall consisting of complex polysaccharides such as chitin, glucans and mannan. Among 70,000 or so species of fungi, only a small numbers are pathogenic for humans. However, the fungi can cause, sometimes, serious life-threatening illness. The fungi can exist as:

1. Single cells (yeasts), which can easily be phagocytosed because of small size.
2. Long sender branching hyphae, which require extracellular killing processes.

Some pathogenic fungi exist in nature as an infectious mould (hyphal form) and invade tissue as a yeast (or yeast-like form such as spherules and endospores). These fungi are known as dimorphic fungi. Both the phases possess important virulent determinants and pose different problems in the immune system.

**Table 18.2** Host immune responses to bacterial infection

| Infection process | Host defence | Bacterial evasion mechanisms |
|---|---|---|
| Attachment to host cells | Blockage of attachment by secretory | Secretion of proteases that cleave secretory IgA antibodies, IgA dimers (*Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*) |
| | | Antigenic variation in attachment structures (pill of *N. gonorrhoeae*) |
| Cell-mediated | Phagocytosis (Ab and C3b-mediated opsonization) | Production of surface structures (polysaccharide capsule, M protein, fibrin coat) that inhibit phagocytic cell |
| | | Mechanisms for surviving within phagocytic cells induction of apoptosis in macrophages (*Shigella flexneri*) |
| | Complement-mediated lysis and localized inflammatory response | Generalized resistance of gram-positive bacteria to complement-mediated lysis |
| | | Insertion of membrane attack complex prevented by long side chain in cell wall LPS (some gram-negative bacteria) |
| Invasion of host tissues | Ab-mediated agglutination | Secretion of elastase that inactivates C3a and C5a (*Pseudomonas*) |
| Toxin-induced damage to host cells | Neutralization of toxin by antibody | Secretion of hyaluronidase, which enhances bacterial invasiveness |

Although, some fungal infections can occur in healthy individuals, the immunocompromised subjects [patients with untreated acquired immunodeficiency syndrome (AIDS), patients undergoing cancer therapy, transplant recipient with immunosuppressive therapy, patients depending on long-term corticosteroid therapy and others] are more prone to suffer from a variety of fungal infections. The immunity against different categories of fungi broadly consists of:

1. Innate immune response.
2. T cell-mediated specific immune responses.

## Innate Immune Response

Intact skin and normal commensal flora plays important role in preventing the entry and colonization of fungi.

Certain antifungal and antibacterial substances such as defensins, mannose-binding lectin (MBL), surface protein ‘A’ and ‘D’, coats over the fungal elements and opsonize them for phagocytosis. Immunity to mycoses is principally cellular, involving neutrophils, macrophages, lymphocytes and probably by NK cells (for extracellular killing). With the possible exception of dermatophytes and *Rhizopus arrhizus,* fungi are immune to antibody-complement-mediated lysis. The phagocytes (neutrophils and macrophages) kill the fungi.

1. Degranulation and release of the toxic materials on to indigestible hyphae.
2. Ingestion of the yeasts or conidia.

The oxidative bursts, following ingestion, play an important role in destruction of fungi. Defect in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, as that occurs in patients with chronic granulomatous disease (CGD), fails to deal with *Aspergillus* species, leading to severe aspergillosis. However, phagocytes from CGD patients, with defective oxygen reduction pathways, are competent enough to kill other yeasts and hyphae with normal efficiency, indicating the role of other mechanisms.

The phagocytic response is dependent on the recognition of (PAMPs) pathogen-associated molecular patterns in the fungal cell wall by either soluble or cell bound pattern recognition molecules. Toll-like receptor 2 (TLR2) recognizes fungal phospholipid mannan of *Candida albicans,* yeasts and *A. fumigatus* hyphae and conidia. TLR4/CD14 recognizes *C. albicans*, *A. fumigatus* and glucuronoxylomannan capsule of *C. neoformans.*

## T Cell-mediated Specific Immune Response

Most fungi are highly immunogenic. They induce antibody production, as well as T CMI, which can be detected by serology and skin test (delayed hypersensitivity), respectively. Antibodies are seldom protective. Considerable evidence suggests that $T_h1$-macrophage activity plays dominant role in eliminating fungal pathogens.

Immunity against most pathogenic fungi (including dermatophytes and most systemic mycoses such as *C. neoformans, H. capsulatum,* etc. but not *Aspergillus* species) is dependent on T CMI particularly, $CD4^+$-$T_h1$ cell secreting cytokine (IFN-γ).

In some fungal infection, such as paracoccidioidomycosis, the type of immune response depends upon the severity of the lesion. In mild paracoccidioidomycosis, $T_h1$ response dominates. On the other hand, in severe disseminated paracoccidioidomycosis, the $T_h1$ response is replaced by $T_h2$ response with high level of $T_h2$ cytokines (IL-4, IL-10) and eosinophils. A reduction of IFN-γ (with concomitant increase level of IL-10) is also a marker of impaired immunity in systemic mycosis such as *C. albicans* and neutropenia-associated aspergillosis.

## Evasion Strategies

Many fungi have evolved the ways to circumvent, some of the host defense for their survival:

1. *Cryptococcus neoformans*, ordinarily, inhibits phagocytosis because of its polysaccharide capsule, but can be overcome by the opsonic effect of complement and antibody.
2. Dermatophytes suppress host T cell responses and delay the cell-mediated destruction.
3. *Histoplasma capsulatum,* an obligatory intracellular pathogen, evades killing by macrophage by entering through CR3 receptor and altering the normal pathway of phagosome maturation.

## STUDY QUESTIONS

### Essay Questions

1. Discuss the effector mechanism involved in the elimination of parasites from the host.

2. Briefly discuss the various mechanisms, how the parasites evade the host immune system and established infections.
3. Discuss, briefly the humoral and cell-mediated immune responses to viruses.
4. How viruses evade the host defense mechanisms?
5. Discuss the host immune responses to bacterial infections.
6. Discuss the host immune responses to fungal infections.

### Short Notes

1. Role of eosinophils in parasitic infection.
2. Immunopathological consequences of parasitic infections.
3. Immunity againt intracellular bacteria.
4. Mucosal immunity against viral infections.
5. T cell-mediated immune response against fungal infections.

## SUGGESTED READING

1. Black JG. Microbiology: Principles and applications, 3rd edition. USA: Printice Hall College div; 1996.
2. Coligan JE, Kruisbeek AM, Margulies DH, et al. Current Protocols in Immunology. New York: Wiley; 1997.
3. Daniel P Stites. Basic and Clinical Immunology, 8th edition. USA: Lange (Medical Book); 2007.
4. Gerald L Mandell, John E Bennett, Raphael Dolin. Principles and Practice of Infectious Diseases (Volume 1 and 2), 5th edition. 2000.
5. Goldsby RA, Kindt Thomas J, Osborn Barbara A Kuby. Immunology, 6th edition. New York: WH Freeman and Company; 2007.
6. Jawetz. Melnick and Adelberg's Medical Microbiology, 25th edition. USA: McGraw Hill, Lange Basic Science; 2010.
7. Male David, Brostoff Jonathan, Roitt Ivan, et al. Immunology, 7th edition; 2006.
8. Tortora Gerard J, Funke Berdel R. Case christine L. Microbiology: an introduction, 10th edition. USA; 1998.
9. Thao Doan, Roger Melvold, Susan Viselli, et al. Lippincott's illustrated reviews: Immunology. 1st Indian print. Baltimore, USA: Lippincott Williams and Wilkins; 2008.

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